Biochemical and Biophysical Research Communications 411 (2011) 168–174

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Decorin gene expression and its regulation in human keratinocytes

Cristina Velez-DelValle, Meytha Marsch-Moreno, Federico Castro-Muñozledo, Walid Kuri-Harcuch *

Department of Cell Biology, Centro de Investigación y de Estudios Avanzados del IPN, Apdo. Postal 14-740, México D.F. 07000, Mexico

ARTICLE INFO

Article history:
Received 17 June 2011
Available online 23 June 2011

Keywords:
Epidermal keratinocytes
Terminal differentiation
Decorin
Gene expression
Psoriasis

ABSTRACT

In various cell types, including cancer cells, decorin is involved in regulation of cell attachment, migration and proliferation. In skin, decorin is seen in dermis, but not in keratinocytes. We show that decorin gene (*DCN*) is expressed in the cultured keratinocytes, and the protein is found in the cytoplasm of differentiating keratinocytes and in suprabasal layers of human epidermis. RT-PCR experiments showed that *DCN* expression is regulated by pro-inflammatory and proliferative cytokines. Our data suggest that decorin should play a significant role in keratinocyte terminal differentiation, cutaneous homeostasis and dermatological diseases.

1. Introduction

The small leucine-rich proteoglycan (SLRP) decorin, is part of mammalian extracellular matrices, and it is comprised of a protein core of 45 kDa covalently bound to one or more glycosaminoglycan side chains [1]. It has a dominant structural central domain containing 6–10 leucine-rich repeats (LRR), flanked by N-terminal and C-terminal regions with conserved cysteine residues [2]. Decorin interacts with a variety of proteins involved in regulation of cell attachment [3], cell migration [4], and it inhibits proliferation in several cell types [5–7], including, neoplastic cells [6,8]. *DCN* expression in endothelial cells, hepatocytes, fibroblasts or macrophages is related to terminal differentiation [7,9,10].

Decorin interacts with the epidermal growth factor (EGF) receptor ErbB2 [11] and TGFβ [5,12,13], cytokines with an important function on proliferation, migration, and differentiation of human keratinocytes. Decorin is found in extracellular dermal components of skin, but not in contact with the epidermal keratinocytes. We previously described that fibromodulin is found in cultured human epidermal keratinocytes (cHEK), and in human epidermis in vivo [14]. Here, we determined the presence and localization of decorin in human keratinocytes and human skin. We explored its mRNA expression, protein levels and regulation by various proliferative and pro-inflammatory cytokines.

2. Materials and methods

2.1. Materials

Recombinant human EGF was from Mallinckrodt (Chesterfield, MO), other cytokines and FITC-streptavidin were from Upstate (Lakeplacid, NY). Recombinant human decorin was from R&D (Minneapolis, MN). Rabbit polyclonal antibody against decorin was from Santa Cruz (Santa Cruz, CA). ZyMAX™ HRP anti-rabbit antibody was from Zymed (Invitrogen, CA), and biotinylated antibody against rabbit IgG was from PIERCE (USA). All other reagents were analytical grade.

2.2. Culture of human epidermal keratinocytes

HEK, strain HE-123, were isolated from newborn foreskin. For experiments, 3rd to 5th passage keratinocytes were cultured with 3T3 feeder cells in DMEM-F12 (3:1) [23–26]. Human dermal fibroblasts (strain hFIB-132), isolated by us from a human skin biopsy, were plated in DMEM-F12, as above. All cultures were incubated at 37 °C in a humidified incubator equilibrated with 10% CO_2, and medium was changed every other day.

2.3. RNA Extraction, RT-PCR and sequencing

Total RNA was extracted with Trizol™ reagent (Invitrogen, CA), its integrity was verified by denaturing agarose electrophoresis and used for cDNA synthesis with oligo (dT)-primers. Reverse transcription was carried out with Super Script II (Invitrogen, CA). Conventional PCR amplification was done with Taq DNA Polimerase (Invitrogen, CA). The amplified products were cloned with the

* Corresponding author. Fax: +11 52 55 5747 3393.
E-mail address: walidkuri@gmail.com (W. Kuri-Harcuch).

0006-291X/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2011.06.122

TOPO TA Cloning kit into pCR2.1 (Invitrogen, CA). The insert was sequenced in ABI PRISMTM 310 DNA Sequencer (Perkin-Elmer) with the Big Dye Terminator kit v3.1 (Perkin-Elmer) using M13 and T7 sequencing primers and specific primers. Sequencing data were compared with the published sequences using BLASTN 2.2 [27] of The National Cancer Biotechnology Information Center. Real time PCR was carried out in triplicate experiments with samples treated with DNAse I amplification grade (Invitrogen,CA). cDNA was synthesized as described above and the PCR step was carried out using the LightCycler FastStart DNA Master PLUS SYBR Green I (Roche) in the ABI 7500. To determine the absolute number of DNA copies, we produced a standard curve that related DNA copy number with the amount of fluorescence. We used the previously cloned and sequenced decorin plasmid, and 5′TCCTGAAGAGTT TTGGGCATTTT3′ as a reverse primer which anneals to base 494 of exon 2, and generates a 471 bp product internal to the 1200 bp sequence inserted into the plasmid. The authenticity of the PCR product was verified by melting curve analysis, and agarose gel electrophoresis. The equivalence of the cDNA input was assessed by determination of GAPDH levels from the same samples.

2.4. Immunostaining

HEK were cultured on glass coverslips and fixed with 3.5% (w/v) para-formaldehyde in phosphate-buffered saline (PBS) or by methanol immersion. They were incubated with antibody against decorin, and then stained with a biotinylated secondary antibody and streptavidin-FITC or streptavidin-TxRd. Actin was detected with rhodamine-phalloidin (Molecular Probes). Slides were mounted in Vectashield fluorescent mounting medium. Immunodetection in cultured epithelia or skin biopsies was carried out in 8 μm cryosections embedded with tissue freezing medium (Jung). Epithelia were detached from the culture dish using Dispase II (2.5 mg/ml). Tissue sections of normal and psoriatic skin in silanized glass slides were air-dried and hydrated in PBS for 5 min prior to antibody recognition. Figures were prepared using Adobe Photoshop® without changing the original number of pixels.

2.5. Western blot analysis

Total proteins from HEK or human fibroblasts cultures were electrophoresed in 12.5% SDS–PAGE, transferred to nitrocellulose paper, and detected with anti-decorin as primary antibody, and revealed with horseradish peroxidase (HRP) anti-rabbit IgG. The inmunostained proteins were visualized using luminol™reagent (Santa Cruz, CA). Recombinat human decorin (R&D) was used as a positive control. Actin detection was carried out with an anti-actin antibody that was a generous gift of Dr. Manuel Hernández Hernández.

3. Results

3.1. Decorin in cultured human epidermal keratinocytes and in human epidermis in vivo

We studied the distribution of decorin in cHEK with an antibody against the human protein. Decorin was inmunodetected in basal and stratified keratinocytes, but staining was much less intense

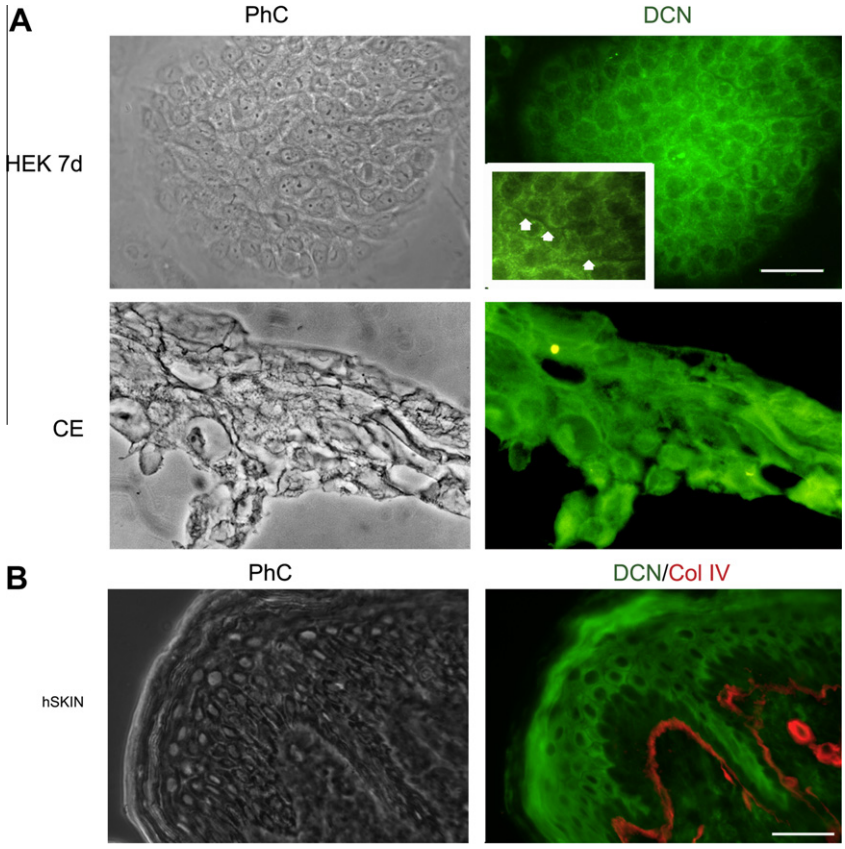

Fig. 1. Immunolocalization of decorin in cHEK and human skin. (A, upper panel) Seven day HEK growing colonies were immunostained with antibodies against human decorin; arrowheads show the basal cells of the proliferative rim are not stained for decorin. (A, lower panel) In 8 μm cryosections of confluent cultured epidermal sheets; decorin is seen in the cytoplasm of keratinocytes. (B) Cryosections of normal human skin showing staining for decorin at suprabasal cell layers but not in the basal cells adjacent to the basal lamina, which was immunostained with an anti-collagen IV antibody. Scale bar = 50 μm.

in the cells of the proliferative rim (Fig. 1A). Remarkably, the staining pattern showed that the protein seemed to be localized intracytoplasmically and undetected in the intercellular space, as it is seen by the dark boundary between immunostained keratinocytes of the growing colonies (Fig. 1A, arrowheads). In cryosections of confluent epithelium it is seen in the cytoplasm (Fig. 1A). In frozen sections from adult human skin, we localized decorin at suprabasal keratinocytes and in stratum corneum, but not in basal cells (Fig. 1B). We also localized decorin in the cytoplasm of the keratinocytes as it was seen in cultures.

By confocal microscopy, we confirmed the intracellular localization of decorin. Double inmunostaining for decorin and actin showed that both proteins were at the same focal plane in each 0.2 μm optical section, but decorin had a punctuate cytoplasmic distribution, not apparently structured as filaments (Fig. 2A). We did not see any association of decorin with actin (Fig. 2A), or with keratins or tubulin (not shown). By double hybridization techniques, it was suggested that filamin, an intracellular protein that cross-links actin, would interact *in vitro* with the LRR motif of decorin [15]. Our immunolocalization and confocal microscopy experiments showed that decorin and filamin, were intracytoplasmic, although they did not co-localize (Fig. 2B), suggesting that the LRR motif of decorin does not interact with filamin *in vivo*.

Western blot of proteins from 2 days post-confluent cHEK that had dislodged the 3T3 feeders cells, showed that decorin was found in total cell extracts but not in extracellular matrix extracts, suggesting that decorin is secreted at undetectable amounts, or not secreted, into the culture medium (Fig. 3A). We treated cHEK for 48 h with Brefeldin-A, which inhibits protein transport in Golgi apparatus, leading to accumulation of proteins inside endoplasmic reticulum [16]. Immunostaining of cHEK treated with Brefeldin-A, showed that the distribution pattern of decorin was not modified (data not shown). Together these results support the observation that decorin is mainly an intracellular protein in human keratinocytes.

3.2. DCN is expressed in cultured human epidermal keratinocytes

We carried out PCR amplification with total RNA from growing (7 day), or 2 days post-confluent (12 day) cHEK. We used specific primers to exons 1 and 10 of the human *DCN*, and obtained a 1200 bp fragment for the open reading frame (Fig. 3B, lanes 5 and 6). As positive control, we identified the same amplification product from total RNA from hFIB-132 (Fig. 3B, lane 4), but it was absent from the RNA of murine 3T3-feeder cells (Fig. 3B, lane 3). The amplification fragment for decorin from cHEK was cloned and sequenced, showing a complete match with the published mRNA sequence for the corresponding human gene (Gene Bank reference NM_133503), indicating that decorin is expressed in cHEK. Quantitative real time PCR amplification showed that *DCN* mRNA levels were similar in both growing and confluent keratinocytes (Fig. 3C), but in any case lower than in human fibroblasts.

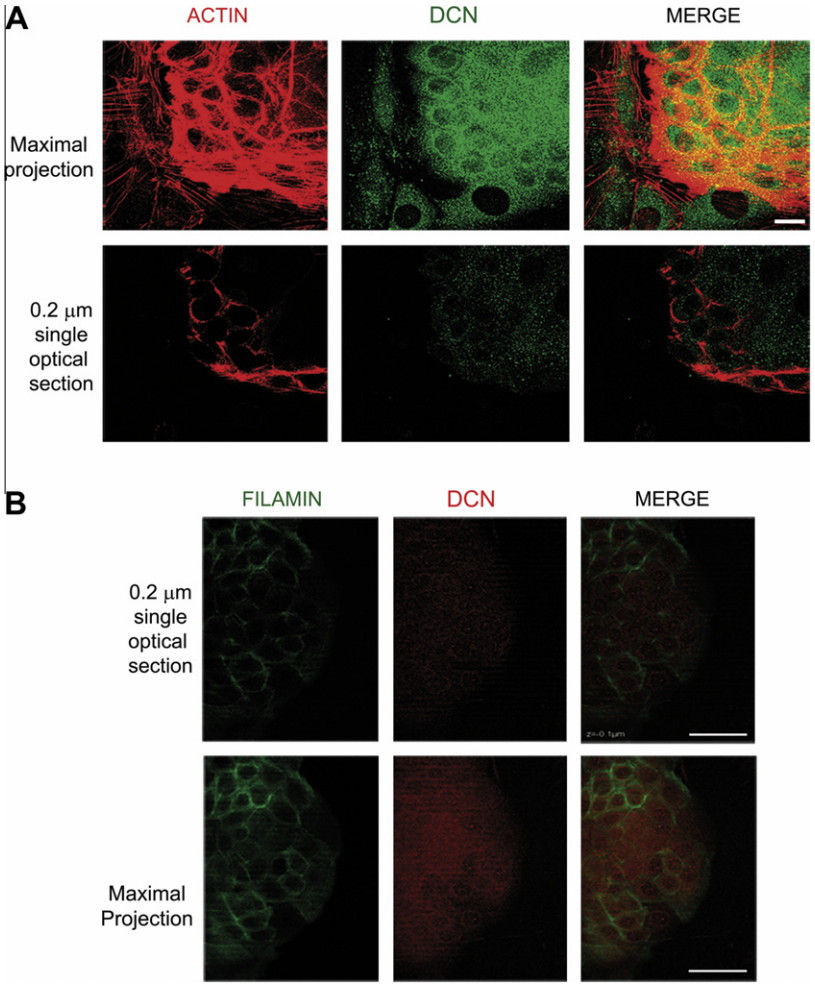

Fig. 2. Intracellular distribution of decorin analyzed with confocal microscopy. (A) Decorin was immunostained in 7 day HEK growing colonies; actin microfilaments were detected using rhodamine-phalloidin. (B) Cells were double stained for decorin and with an anti filamin antibody. (A) Scale bar = 20 μm, (B) scale bar = 50 μm.

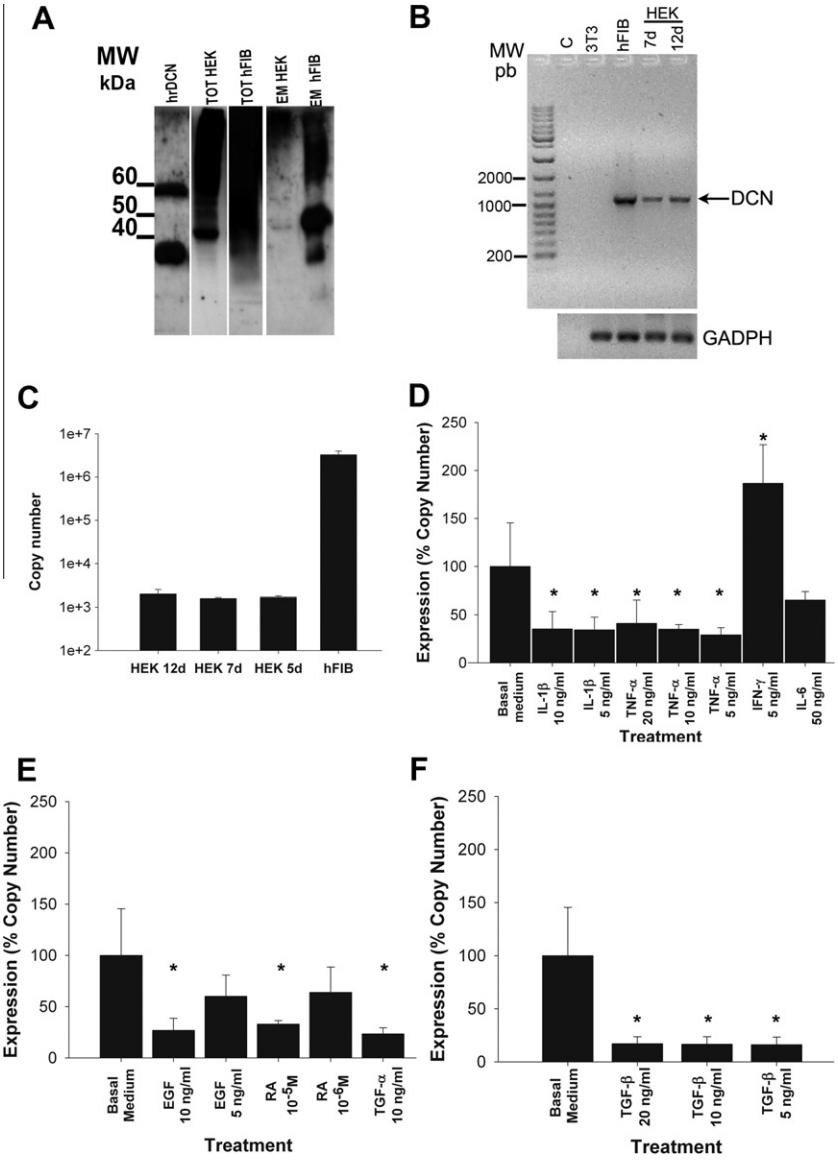

Fig. 3. Detection of decorin protein and mRNA, and effect of cytokines on decorin mRNA expression. (A) Immunoblot analysis of decorin. (Lane 1) human-recombinant decorin; (lane 2) total cell extracts from HEK or (Lane 3) human fibroblast (hFib) cell cultures. (Lane 4) extracellular matrix extracts from cHEK or (Lane 5) cultured hFib, showing almost no signal from HEK cells. (B) End point PCR for decorin encoding mRNA. The 1150 bp product corresponds to the ORF of human decorin. (B, lower panel) GAPDH mRNA was used as an amplification control. (C) Real time PCR amplification of decorin mRNA expression in total RNA from HEK at different times in culture, and in confluent hFib. (D, E, and F) Confluent cultures of HEK were exhaustively rinsed, and changed to basal culture medium alone or containing the specified concentrations of (D) inflammatory cytokines, (E) proliferative cytokines or (F) TGFβ. Treatment with cytokines was maintained 48 h, and cultures were harvested. Results are average ± SD of triplicate cultures from two independent experiments. *$P < 0.05$.

3.3. DCN expression in cHEK is modulated by cytokines

During inflammation, decorin is induced in endothelial cells by cytokines [10,17]. We looked into *DCN* expression after treatment of confluent cHEK, for 48 h, with inflammatory cytokines. Interleukin 1β (IL-1β) and TNF-α inhibited the expression of *DCN* in about 50%, and Interleukin 6 (IL-6) did not inhibit it significantly, as compared with non-treated cultures (Fig. 3D). Interferon gamma (IFN-γ) significantly increased, by 2-fold, the expression levels of *DCN* .

We also assayed the effect of EGF and transforming growth factor alpha (TGF-α), that promote cHEK proliferation. Both cytokines reduced the expression levels of *DCN* in about 75% as compared to cHEK maintained with basal medium (Fig. 3E). Retinoic acid (RA), that promotes cHEK proliferation [18,19], also decreased *DCN* expression (Fig. 3E). Treatment with these agents, which have a general effect in stimulating proliferation of HEK, promoted a significant decrease in the expression levels of *DCN* (Fig. 3E).

After treatment of cHEK with 5–20 ng/ml TGFβ, we found that it reduced *DCN* expression levels in 80% (Fig. 3F). By immunoblotting of total protein extracts from parallel cultures, we did not find any changes in the relative amounts of decorin (Fig. Sup). This result could be explained by an intracytoplasmic decorin half-life longer than 48 h, which is not known. Another explanation is that there is a large amount of protein in the upper part of the western blot for decorin, comprising a broad range of molecular weights (Fig. Sup). Since this could be due to the proteoglycan forms of decorin, it is difficult to determine any precise changes in the amount of protein. These possibilities might explain the apparent contradictory data between mRNA expression and protein content.

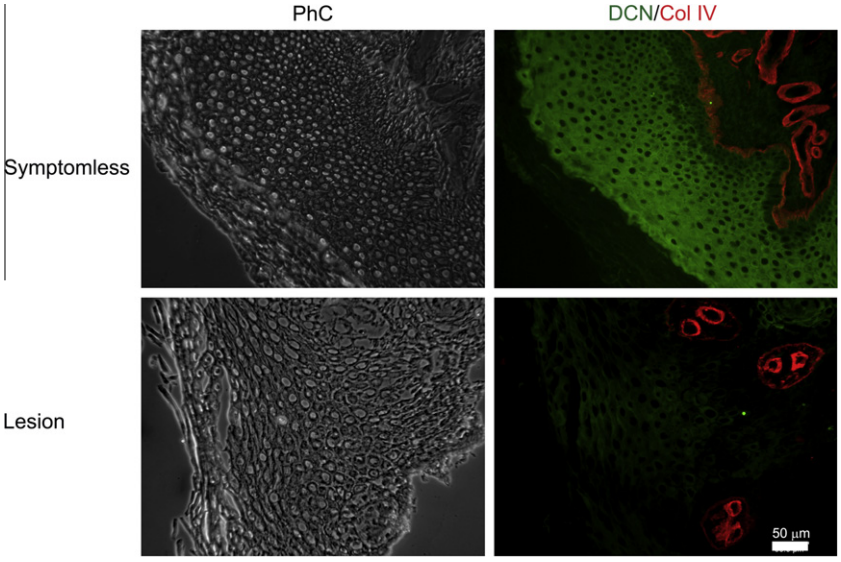

Fig. 4. Decorin expression in psoriatic skin. 8 µm cryosections were obtained from symptomless and plaques of psoriatic human skin, and immunodetected against decorin and Collagen IV. Scale bar = 50 µm.

3.4. Decorin is altered in psoriatic lesions

IL-1α, IL-6, and TNF-α are increased in plaques of psoriatic skin [20]. Since we found that TNF-α regulates *DCN* expression in keratinocytes (see above), we immunostained psoriatic skin lesions to detect decorin. Results showed the disrupted pattern of epidermal stratification and basement membrane organization characteristic of psoriatic skin [21,22] (Fig. 4). Decorin immunostaining of psoriatic lesions showed a lower intensity and more altered intracytoplasmic arrangements, in comparison with non-lesion skin from the same patient (Fig. 4).

4. Discussion

To our knowledge, this is the first report on the identification and localization of decorin, and on the regulation of *DCN* expression by cytokines in human keratinocytes. Previously, we described the expression of the fibromodulin gene in cHEK and the presence of the corresponding protein in both cHEK and normal human epidermis [14]. Here, we describe decorin in human keratinocytes, but with a different localization in the epidermal compartment as compared to fibromodulin. Other reports did not show these proteoglycans in cultured epithelial cells. We think that our approach has two main advantages that can explain the differences with previous reports [28]. First, co-cultivation of human epidermal keratinocytes with 3T3 feeder cells supports the proliferative potential of keratinocytes, the expression of their differentiation markers, and the deposition of a more complete extracellular matrix [25]. Second, we used more sensitive methods such as RT-PCR and real time RT-PCR for detection of mRNAs, than the use of Northern blot analysis [29]. When we were carrying out this work, small amounts of *DCN* were seemingly detected in human epidermis by combining laser capture micro-dissection and real time RT-PCR [30], but its localization, its distribution in the epidermal cell layers, its regulation and function in the keratinocyte were not addressed. By immunostaining and confocal microscopy analysis, we localized decorin intracellulary in both cHEK and keratinocytes in human skin biopsies. Also, we found the presence of decorin in total cell extracts but not in the extracellular matrix extracts.

Localization of decorin in the cytoplasm of keratinocytes, mainly in the suprabasal epidermal layers in cultures and in skin sections, strongly suggests that this protein is most likely involved in terminal differentiation of keratinocytes. However, its specific role remains to be elucidated. Some of its structural characteristics, one repeat of two glutamines and two repeats of two lysines in its primary structure, and its suprabasal localization in epidermis, would suggest that decorin might be a substrate for TGase, which catalyzes cornified envelope formation during epidermal terminal differentiation [31,32]. Involucrin, another TGase substrate for cross-linking in cornified envelop formation [32,33], show a distribution which correlates with the observed distribution of decorin in cHEK and human epidermis (see Fig. 1B). However, disruption of TGase activity by cadaverine in the cHEK did not modify the levels of *DCN* mRNA (Results not shown). But, is clear that decorin is found in keratinocytes during their terminal differentiation, suggesting a role during this process, which our experiments have not yet elucidated.

In an attempt to gain an insight in decorin possible role in epithelial cells, we studied the expression of *DCN* in cHEK treated with inflammatory, proliferative and pro-fibrotic cytokines. Our results showed that the inflammatory cytokines IL-1β, and TNF-α decreased *DCN* expression, but in contrast, IFN-γ increased its expression by about 2-fold. These results suggest that inflammatory cytokines modulate decorin synthesis under various conditions. If decorin is involved in keratinocyte terminal differentiation, a decrease on its gene expression might reduce terminal differentiation and consequently enhance epidermal proliferation. This could be part of the mechanism in hyper proliferative diseases of the skin, such as psoriasis, in which we observed a diminished staining for decorin. Psoriasis is characterized by an increased synthesis of inflammatory cytokines, such as TNF-α and IL-1β [20], and epidermal hyperproliferation. These observations are in agreement with the data we obtained with TNF-α and the proliferative cytokines, EGF and TGF-α. These cytokines are over expressed in psoriasis [20]. *DCN* expression in the cHEK is reduced by these three cytokines. It is suggestive that a decrease in the expression of some of the genes involved in terminal differentiation, whether by inflammatory conditions or by growth factors, should lead to a state of hyperproliferation in the epidermis, which correlates with the relative content of decorin observed in tissue sections of psoriatic lesions.

TGFβ promotes the basal phenotype of epidermal keratinocytes, as determined by the transcriptional induction of the basal keratin

pair K5/K14 [34]. Our data showed a decrease in *DCN* expression elicited by TGFβ. This correlates with a decrease in terminal differentiation of keratinocytes. It also should be considered that *DCN* promoter has a region that might be negatively regulated by TGFβ [35]. This cytokine is also up-regulated in the psoriatic lesion [36].

High levels of TNF-α have been detected in psoriatic plaques and they correlate with the severity of the disease [37]. These data are in agreement with our results showing that TNF-α decreases the expression of *DCN* in keratinocytes (Fig. 4B). The only tested cytokine that induced the expression of *DCN*, was IFN-γ, which has a stimulatory effect in the expression of involucrin [38] a major member of the cornified envelope. It is also of relevance that IFN-γ, the cytokine that increased *DCN* levels in keratinocytes (Fig. 4A), inhibits proliferation of psoriatic keratinocytes and it is used in its therapy [39].

We were unable to precisely identify the function of decorin, remaining puzzling its role in human epidermis. However, based on the regulation of *DCN* expression by cytokines, the abundance of the protein, and its intracytoplasmic localization, we can suggest that decorin might have a possible role during terminal differentiation of keratinocytes, and in hyperproliferative diseases of the skin. Decorin has an anti-oncogenic function, and it is not frequently expressed in transformed epithelial cells [11]. These data and our results strongly suggest that decorin would have a significant function in skin homeostasis, terminal differentiation of epithelial cells, both diploid and neoplastic, that should warrant further investigation in epithelial cells to unravel its function and mechanism of action.

Acknowledgments

This work was supported in part by CONACyT Grant 104350. and by the Grant PICDS08-8 from ICyT D.F. C. Velez-DelValle is supported by a postdoctoral fellow from Grant PICDS08-8 from ICyT D.F. We thank Biol. Alicia Beltrán Langarica for their technical assistance. We also thank Ms. María Elena Rojano for her secretarial assistance and Mr. Alberto Rodriguez and Mrs. Columba Guadarrama for preparing all materials.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2011.06.122.

References

[1] L. Kjellen, U. Lindahl, Proteoglycans: structures and interactions, Annu. Rev. Biochem. 60 (1991) 443–475.
[2] P.A. McEwan, P.G. Scott, P.N. Bishop, J. Bella, Structural correlations in the family of small leucine-rich repeat proteins and proteoglycans, J. Struct. Biol. 155 (2006) 294–305.
[3] B. Merle, L. Malaval, J. Lawler, P. Delmas, P. Clezardin, Decorin inhibits cell attachment to thrombospondin-1 by binding to a KKTR-dependent cell adhesive site present within the N-terminal domain of thrombospondin-1, J. Cell Biochem. 67 (1997) 75–83.
[4] B. Merle, L. Durussel, P.D. Delmas, P. Clezardin, Decorin inhibits cell migration through a process requiring its glycosaminoglycan side chain, J. Cell Biochem. 75 (1999) 538–546.
[5] Z. Zhang, X.J. Li, Y. Liu, X. Zhang, Y.Y. Li, W.S. Xu, Recombinant human decorin inhibits cell proliferation and downregulates TGF-beta1 production in hypertrophic scar fibroblasts, Burns 33 (2007) 634–641.
[6] Y. Yamaguchi, E. Ruoslahti, Expression of human proteoglycan in Chinese hamster ovary cells inhibits cell proliferation, Nature 336 (1988) 244–246.
[7] J. Xaus, M. Comalada, M. Cardo, A.F. Valledor, A. Celada, Decorin inhibits macrophage colony-stimulating factor proliferation of macrophages and enhances cell survival through induction of p27(Kip1) and p21(Waf1), Blood 98 (2001) 2124–2133.
[8] M. Santra, D.M. Mann, E.W. Mercer, T. Skorski, B. Calabretta, R.V. Iozzo, Ectopic expression of decorin protein core causes a generalized growth suppression in neoplastic cells of various histogenetic origin and requires endogenous p21. An inhibitor of cyclin-dependent kinases, J. Clin. Invest. 100 (1997) 149–157.
[9] J.G. Tralhao, L. Schaefer, M. Micegova, C. Evaristo, E. Schonherr, S. Kayal, H. Veiga_Fernandes, C. Danel, R.V. Iozzo, H. Kresse, P. Lemarchand, *In vivo* selective and distant killing of cancer cells using adenovirus-mediated decorin gene transfer, FASEB J. 17 (2003) 464–466.
[10] E. Schonherr, B.C. O'Connell, J. Schittny, H. Robenek, D. Fastermann, L.W. Fisher, G. Plenz, P. Vischer, M.F. Young, H. Kresse, Paracrine or virus-mediated induction of decorin expression by endothelial cells contributes to tube formation and prevention of apoptosis in collagen lattices, Eur. J. Cell Biol. 78 (1999) 44–55.
[11] M. Santra, I. Eichstetter, R.V. Iozzo, An anti-oncogenic role for decorin. Down-regulation of ErbB2 leads to growth suppression and cytodifferentiation of mammary carcinoma cells, J. Biol. Chem. 275 (2000) 35153–35161.
[12] N. Abdel-Wahab, S.J. Wicks, R.M. Mason, A. Chantry, Decorin suppresses transforming growth factor-beta-induced expression of plasminogen activator inhibitor-1 in human mesangial cells through a mechanism that involves Ca2+-dependent phosphorylation of Smad2 at serine-240, Biochem. J. 362 (2002) 643–649.
[13] Y.F. Shi, Q. Zhang, P.Y. Cheung, L. Shi, C.C. Fong, Y. Zhang, C.H. Tzang, B.P. Chan, W.F. Fong, J. Chun, H.F. Kung, M. Yang, Effects of rhDecorin on TGF-beta1 induced human hepatic stellate cells LX-2 activation, Biochim. Biophys. Acta 1760 (2006) 1587–1595.
[14] C. Velez-DelValle, M. Marsch-Moreno, F. Castro-Munozledo, Y.J. Bolivar-Flores, W. Kuri-Harcuch, Fibromodulin gene is expressed in human epidermal keratinocytes in culture and in human epidermis *in vivo*, Biochem. Biophys. Res. Commun. 371 (2008) 420–424.
[15] K. Yoshida, Y. Suzuki, E. Honda, K. Amemiya, T. Nakatani, M. Ebina, K. Narumi, K. Satoh, H. Munakata, Leucine-rich repeat region of decorin binds to filamin-A, Biochimie 84 (2002) 303–308.
[16] R.D. Klausner, J.G. Donaldson, J. Lippincott-Schwartz, Brefeldin A: insights into the control of membrane traffic and organelle structure, J. Cell Biol. 116 (1992) 1071–1080.
[17] L. Nelimarkka, H. Salminen, T. Kuopio, S. Nikkari, T. Ekfors, J. Laine, L. Pelliniemi, H. Jarvelainen, Decorin is produced by capillary endothelial cells in inflammation-associated angiogenesis, Am. J. Pathol. 158 (2001) 345–353.
[18] B. Chapellier, M. Mark, N. Messaddeq, C. Calleja, X. Warot, J. Brocard, C. Gerard, M. Li, D. Metzger, N.B. Ghyselinck, P. Chambon, Physiological and retinoid-induced proliferations of epidermis basal keratinocytes are differently controlled, EMBO J. 21 (2002) 3402–3413.
[19] S. Gibbs, C. Backendorf, M. Ponec, Regulation of keratinocyte proliferation and differentiation by all-trans-retinoic acid, 9-cis-retinoic acid and 1, 25-dihydroxy vitamin D3, Arch. Dermatol. Res. 288 (1996) 729–738.
[20] P.C. van de Kerkhof, The evolution of the psoriatic lesion, Br. J. Dermatol. 157 (2007) 4–15.
[21] M.R. Mondello, L. Magaudda, S. Pergolizzi, A. Santoro, M. Vaccaro, L. Califano, S.P. Cannavo, B. Guarneri, Behaviour of laminin 1 and type IV collagen in uninvolved psoriatic skin. Immunohistochemical study using confocal laser scanning microscopy, Arch. Dermatol. Res. 288 (1996) 527–531.
[22] R. Fleischmajer, K. Kuroda, R. Hazan, R.E. Gordon, M.G. Lebwohl, A.N. Sapadin, F. Unda, N. Iehara, Y. Yamada, Basement membrane alterations in psoriasis are accompanied by epidermal overexpression of MMP-2 and its inhibitor TIMP-2, J. Invest. Dermatol. 115 (2000) 771–777.
[23] J. Bolivar-Flores, E. Poumian, M. Marsch-Moreno, G. Montes de Oca, W. Kuri-Harcuch, Use of cultured human epidermal keratinocytes for allografting burns and conditions for temporary banking of the cultured allografts, Burns 16 (1990) 3–8.
[24] F. Castro-Muñozledo, C. Valencia-Garcia, W. Kuri-Harcuch, Cultivation of rabbit corneal epithelial cells in serum-free medium, Invest. Ophthalmol. Vis. Sci. 38 (1997) 2234–2244.
[25] J.G. Rheinwald, H. Green, Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinizing colonies from single cells, Cell 6 (1975) 331–343.
[26] F. Castro-Muñozledo, M. Hernandez-Quintero, M. Marsch-Moreno, W. Kuri-Harcuch, Cultivation, serial transfer, and differentiation of epidermal keratinocytes in serum-free medium, Biochem. Biophys. Res. Commun. 236 (1997) 167–172.
[27] S.F. Altschul, T.L. Madden, A.A. Schaffer, J. Zhang, Z. Zhang, W. Miller, D.J. Lipman, Gapped BLAST and PSI-BLAST: a new generation of protein database search programs, Nucleic Acids Res. 25 (1997) 3389–3402.
[28] P. Bianco, L.W. Fisher, M.F. Young, J.D. Termine, P.G. Robey, Expression and localization of the two small proteoglycans biglycan and decorin in developing human skeletal and non-skeletal tissues, J. Histochem. Cytochem. 38 (1990) 1549–1563.
[29] K. Reue, MRNA quantitation techniques: considerations for experimental design and application, J. Nutr. 128 (1998) 2038–2044.
[30] K. Lochner, A. Gaemlich, K.M. Sudel, K. Venzke, I. Moll, A. Knott, I. Moll, F. Stab, H. Wenck, O. Doring, M. Bottger, S. Gallinat, Expression of decorin and collagens I and III in different layers of human skin in vivo: a laser capture microdissection study, Biogerontology 8 (2006) 269–282.
[31] S. Jeon, P. Djian, H. Green, Inability of keratinocytes lacking their specific transglutaminase to form cross-linked envelopes: absence of envelopes as a simple diagnostic test for lamellar ichthyosis, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 687–690.
[32] R.H. Rice, H. Green, The cornified envelope of terminally differentiated human epidermal keratinocytes consists of cross-linked protein, Cell 11 (1977) 417–422.

[33] R.H. Rice, M. Mehrpouyan, W. O'Callahan, N.L. Parenteau, A.L. Rubin, Keratinocyte transglutaminase: differentiation marker and member of an extended family, Epithelial. Cell Biol. 1 (1992) 128–137.
[34] C.K. Jiang, M. Tomic-Canic, D.J. Lucas, M. Simon, M. Blumenberg, TGF beta promotes the basal phenotype of epidermal keratinocytes: transcriptional induction of K#5 and K#14 keratin genes, Growth Factors 12 (1995) 87–97.
[35] M. Santra, K.G. Danielson, R.V. Iozzo, Structural and functional characterization of the human decorin gene promoter. A homopurine-homopyrimidine S1 nuclease-sensitive region is involved in transcriptional control, J. Biol. Chem. 269 (1994) 579–587.
[36] I. Flisiak, B. Chodynicka, P. Porebski, R. Flisiak, Association between psoriasis severity and transforming growth factor beta(1) and beta (2) in plasma and scales from psoriatic lesions, Cytokine 19 (2002) 121–125.
[37] P. Ettehadi, M.W. Greaves, D. Wallach, D. Aderka, R.D. Camp, Elevated tumour necrosis factor-alpha (TNF-alpha) biological activity in psoriatic skin lesions, Clin. Exp. Immunol. 96 (1994) 146–151.
[38] H. Takahashi, K. Asano, S. Nakamura, A. Ishida-Yamamoto, H. Iizuka, Interferon-gamma-dependent stimulation of human involucrin gene expression: STAT1 (signal transduction and activators of transcription 1) protein activates involucrin promoter activity, Biochem. J. 344 (Pt 3) (1999) 797–802.
[39] V.B. Morhenn, K. Pregerson-Rodan, R.H. Mullen, G.S. Wood, B.J. Nickoloff, S.A. Sherwin, E.M. Farber, Use of recombinant interferon gamma administered intramuscularly for the treatment of psoriasis, Arch. Dermatol. 123 (1987) 1633–1637.