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Victoria Sarafian · Ralph Jans · Yves Poumay

Expression of lysosome-associated membrane protein 1 (Lamp-1) and galectins in human keratinocytes is regulated by differentiation

Abstract Lysosomes and their components are suspected to be involved in epidermal differentiation. In this study, lysosomal enzyme activities, expression of the lysosome-associated membrane protein 1 (Lamp-1) and expression of the epidermal galectins-1, -3 and -7 were investigated in human keratinocytes cultured at different cell densities (subconfluence, confluence and postconfluence) in order to induce differentiation. Detected by Western blot and immunofluorescence, Lamp-1 expression is transiently upregulated at culture confluence, but reduced at postconfluence. Northern blot analyses performed on subconfluent, confluent and post-confluent cultures of keratinocytes show that Lamp-1 mRNA expression is also upregulated at culture confluence, but downregulated at postconfluence. Measurements of lysosomal enzyme activities indicate a transient upregulation at culture confluence, whereas cathepsins B, C and L are particularly downregulated at postconfluence. Cell density and differentiation of epidermal cells also differentially regulates galectin expression in autocrine cultures. As the expression of galectin-1 mRNA is high in subconfluent cells, it is assumed to be associated with their proliferative state. On the other hand, as the mRNA levels for galectins-3 and -7 are notably upregulated at culture confluence (galectin-7) or at postconfluence (galectin-3), their expression is thought to be related to the differentiated state of keratinocytes. However, we collected evidence by confocal microscopy that galectin-3 and Lamp-1 do not colocalize in vitro in keratinocytes. Altogether, our results suggest that the upregulated Lamp-1 expression at confluence could be involved in keratinocyte differentiation, but apparently not through interaction with galectin-3.

Keywords Keratinocytes · Lysosomes · Lamp-1 · Galectins · Culture confluence

V. Sarafian · R. Jans · Y. Poumay (✉)
Department of Histologie-Embryologie, Facultés Universitaires Notre-Dame de la Paix, 61, Rue de Bruxelles,
5000 Namur, Belgium
E-mail: yves.poumay@fundp.ac.be
Tel.: +32-81-724257
Fax: +32-81-724261

V. Sarafian
Department of Biology, Medical University-Plovdiv,
Plovdiv, Bulgaria

Introduction

Keratinocytes in the basal epidermal layer are proliferative cells, which then leave the cell cycle, migrate into the suprabasal layers and stratify in order to differentiate towards the superficial cornified layer of the epidermis. During this journey, keratinocytes exhibit progressive morphological changes due to programmed terminal differentiation and mature keratinocytes desquamate from the surface of the skin as dead enucleate cells [52]. Epidermal differentiation is a progressive complex phenomenon that depends on the fine regulation of gene expression in keratinocytes. Indeed, many genes are expressed in a coordinated manner during the differentiation of keratinocytes [12, 15, 53]. Beside involucrin, the suprabasal keratins 1 and 10, and other proteins used commonly as differentiation markers (e.g. transglutaminase-1, profilaggrin, loricrin) [13, 15], the involvement of many other genes and their encoded proteins in the development of the mature cornified phenotype of epidermal keratinocytes is increasingly identified.

As a model for in vitro studies of the regulation of differentiation in human epidermal keratinocytes, autocrine cultures of keratinocytes demonstrate initiation of early differentiation when the cell density is high enough to reach confluence of the culture [25, 35, 36], and allow the expression of later markers of differentiation in post-confluent conditions [36, 43]. Thus, autocrine keratinocyte cultures represent a model highly suitable for studying epidermal differentiation.

Lysosomes are intracellular organelles containing acid hydrolases and are responsible for intracellular digestion. Their role in cell differentiation is supported by several studies performed on different cell types

including keratinocytes [5, 9, 45, 57]. A particular importance of lysosomes in keratinocytes has been suggested by the high level of biosynthesis and processing of the lysosomal protein ganglioside G_{M2} activator protein observed in cultured keratinocytes in comparison with fibroblasts [16]. Furthermore, a secretion of lysosomal enzymes from lamellar granules in terminally differentiated keratinocytes has been established [28], and the lysosomal protease cathepsin L was shown to regulate keratinocyte differentiation [46].

The lysosome-associated membrane protein 1 (Lamp-1) is a transmembrane glycoprotein predominantly found in lysosomal membranes. Diverse biological and pathological functions have been proposed for this protein, but its precise role has not been defined yet. Interestingly, it was demonstrated that Lamp-1 is a differentiation marker for mouse mammary epithelial HC11 cells [5]. Hence, the cell surface expression of Lamp-1 on tumour cells [39, 41] and on peripheral blood lymphocytes [24] has suggested that these glycoproteins may play a role as adhesion molecules. Our previous study indicates that Lamp-1 on the surface of tumour cells binds galectin-3, thus possibly promoting tumour invasion and/or metastasis [41]. Galectins are involved in cell proliferation and death, but also in cell adhesion [33], as well as in inflammation, immunity [37] and cancer [30]. Galectin-1 is detected in normal human skin and its gene expression is localized in keratinocytes of the basal and spinous epidermal layers [1]. During the first trimester of human embryogenesis, epidermal galectin-1 expression becomes detectable in basal keratinocytes at 14 weeks, suggesting a possible developmental regulation pattern of the gene [49]. Galectin-1, galectin-3 and galectin-7 expressions have been observed in normal human epidermis [1, 55].

We have recently shown that Lamp-1, and also Lamp-2, are expressed on the surface of keratinocytes treated with ionomycin in order to increase the intracellular calcium concentration [23]. Here, we studied the regulation of Lamp-1 and lysosomes during epidermal differentiation induced in vitro by cell culture density. An upregulation of Lamp-1 at culture confluence, together with differential regulation of the expression of galectins around confluence strongly suggest that lysosomes and galectins may play a role during the differentiation of epidermal keratinocytes.

Materials and methods

Skin samples and culture of normal human keratinocytes

Human adult abdominal skin samples were obtained at plastic surgery (Dr. B. Bienfait, Clinique St. Luc, Namur-Bouge). Samples for microscopy were frozen immediately in Tissue-Tek O.C.T. compound (Sakura) and stored at −80°C until further processing. Keratinocytes were isolated by the trypsin float technique as described [54], and primary cultures were initiated in KGM-2 medium (BioWhittaker, Europe). Keratinocytes harvested from trypsinized primary proliferating sub-confluent cultures were then plated into secondary cultures at 5×10^3 cells/cm^2 in the same medium. All experiments were performed on cells cultured in autocrine conditions [35], which means that, when approximately 40% of the culture substratum was covered by cells, secondary cultures were washed repeatedly with KBM-2 medium prepared by excluding bovine pituitary extract, insulin, transferrin, epinephrine and EGF from the culture medium. Cultures were re-fed every other day with KBM-2 medium until use at culture subconfluence (4 days before the expected confluence), at confluence, or 4 days after confluence, at postconfluence.

Confluence of the cultures induces growth arrest and terminal differentiation of the keratinocytes, characterized by the induction of early epidermal differentiation markers such as the suprabasal keratins 1 and 10 [35]. In post-confluent autocrine keratinocyte cultures, the later marker of differentiation involucrin is upregulated [36].

Immunofluorescent labelling

Normal skin samples coated in O.C.T. were cut at 5 μm in a Microm HM 500 OM cryostat at −20°C, air-dried and fixed on ice with acetone for 4 min. Sections were then washed three times in PBS solution (pH 7.2) and blocked in PBS containing 0.1% bovine serum albumin. Incubation with the primary H4A3 antibody, specific to human Lamp-1 and diluted in the blocking solution, was performed for 1 h at room temperature in a humidified chamber. The mouse H4A3 antibody developed by Drs. J.T. August and J.E.K. Hildreth was obtained from the Developmental Studies Hybridoma Bank maintained at The University of Iowa, Department of Biological Sciences, Iowa City, IA 52242, under contract N01-HD-7-3263 from the NICHD. Incubation with the secondary antibody (fluorescein isothiocyanate (FITC)-conjugated anti-mouse) (Amersham, USA) was for 30 min as above in the dark. Sections incubated either without primary antibodies or with irrelevant mouse antibodies were used as controls. Tissue sections were observed by fluorescent microscopy in a Provis microscope (Olympus, USA).

Immunofluorescent labelling for Lamp-1 and galectin-3 was performed on cultured keratinocytes plated and grown on coverslips. Cells were then fixed and processed as described for frozen sections, or double labelled with the mouse antibody H4A3 as above for Lamp-1, followed by a goat anti-mouse antibody linked to Alexa 568 (red, Molecular Probes, Eugene, OR, USA). Coverslips were also incubated with a rabbit antibody to galectin-3 (Abcam Ltd, Cambridge, UK) followed by a goat anti-rabbit antibody linked to Alexa 488 (green, Molecular Probes, Eugene, OR, USA). The double labelling was then observed on a Leica confocal microscope with both red and green fluorescence combined in order to detect an eventual co-localization.

Western blot analysis

Cell lysates were prepared by scraping cultures into Triton X-100-containing PBS buffer. The protein concentration was determined in each sample and 13 μg of protein from each sample were then resolved by 10% sodium dodecylsulfate–polyacrylamide gel electrophoresis. The proteins were electro-transferred to Immobilon-P membranes (Millipore, Bedford, MA, USA) and the membranes blocked in 3% skimmed milk/0.5% BSA. Lamp-1 protein is detected by incubation for 2 h with H4A3 antibody, followed by three 10 min washes and 1 h incubation with a peroxidase-conjugated anti-mouse antibody.

The final detection was performed with 1:1000 dilutions of horse radish peroxidase-conjugated secondary anti-rabbit or anti-mouse antibodies (DAKO, Hamburg, Germany) and visualized using the BM Chemiluminescence Blotting Substrate (Roche, Penzberg, Germany).

RNA isolation and NORTHERN blot analysis

Poly(A)RNA was isolated and analyzed by Northern blotting as described previously [35]. The probes used to analyze epidermal differentiation are cDNAs specific to the human basal keratin 14 (K14) or specific to the suprabasal keratins 1 (K1) or 10 (K10) [38], and cDNA specific to the human involucrin [11]. The 36B4 cDNA specific to the human acidic ribosomal phosphoprotein PO [27] was used to assure equivalent loading and transfer of RNA.

The probe for the human Lamp-1 was obtained by RT-PCR. Briefly, RNA from human keratinocytes was reverse transcribed to generate first strand cDNA using 5 μM random hexamers, Superscript reverse transcriptase (Life Technologies, Mississauga, ON, Canada) and reaction mix. This cDNA was used as a template for PCR in 20 μl containing 0.25 mM dNTP, 0.5 μM of each primer and two units of Taq DNA polymerase (Life Technologies, Mississauga, ON, Canada) in a buffer containing 20 mM Tris–HCl pH 8.4, 1.5 mM MgCl$_2$ and 50 mM KCl. The reaction conditions involved initial denaturation for 4 min at 94°C, followed by 25 cycles of 0.5 min at 94°C, 0.5 min at 59°C and 1 min at 72°C. The primers used for PCR were TGACACCATCCGTCTGTCTTGG and CATTGTACACAGCGCAGAACAGG (sense and antisense, respectively) designed to amplify a 1,050 bp fragment localized in exon 9 of Lamp-1 [42].

Galectin RNA expression was detected by using human cDNAs specific for galectin-1, galectin-3 and galectin-7 [48]. Relative gene expression was determined by comparison of the specific gene expression with 36B4 expression, based on densitometric measurements obtained with the NIH Image Analysis 1.60 software.

Measurement of lysosomal enzymatic activities

Enzymatic activities were assayed according to the following references: β-glucuronidase [7], α-mannosidase, β-galactosidase and β-glucosidase [34], hexosaminidase [47], cathepsin C [22], cathepsin B and cathepsin L [2], and acid phosphatase [51]. α-glucosidase and α-fucosidase were measured as described for α-mannosidase but using 4-methylumbelliferyl-α-D-glucoside and 4-methylumbelliferyl-α-L-fucoside, respectively, as substrates.
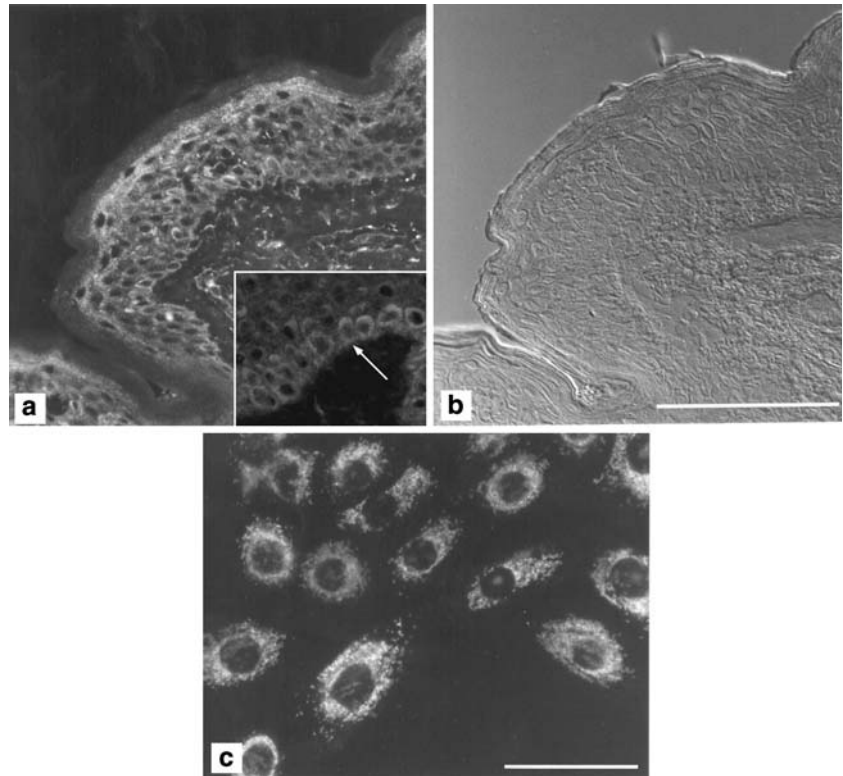
Results

Lamp-1 expression in normal human keratinocytes

Immunofluorescent staining of Lamp-1 on skin sections reveals expression of the protein in cells present in the dermis and in the epidermis (Fig. 1a). A few cells in the basal layer of the epidermis contain some particular staining for Lamp-1 in their cytoplasm localized in a supranuclear position. By the use of silver affinity staining to detect melanin on a tissue section and immunofluorescent staining to localize Lamp-1 in a successive section, we found that Lamp-1 in basal cells co-localizes mainly with melanosomes (data not shown). This observation is in accordance with the lysosomal identity of these organelles [32]. In suprabasal layers, Lamp-1 is present at higher concentration in keratinocytes of the granular layer versus keratinocytes of the spinous layer (Fig. 1a). The epidermal layers are identified by differential interference contrast microscopy (Fig. 1b). The same immunofluorescent staining of Lamp-1 in monolayer cultures of keratinocytes clearly illustrates the elevated number of lysosomes in this cell type (Fig. 1c).

Lysosomal enzymatic activities are upregulated in correlation with the induction of keratinocyte differentiation at culture confluence

Keratinocyte cultures grown in autocrine conditions were collected when still highly proliferating at sub-confluence, when growth-arrested at confluence, or 4 days later at postconfluence in order to measure specific lysosomal activities of several acid hydrolases. The specific activities measured at confluence or postconfluence were compared with the respective specific activities obtained in subconfluent keratinocytes in order to determine ratios of activities in differentiating keratinocytes versus activities in non-differentiating cells (Table 1). The ratios for culture confluence indicate an increase in all lysosomal specific enzymatic activities in differentiating cells, except for cathepsin L, which is decreased. Interestingly, at postconfluence, no further increase of hydrolases activities is detected. Conversely, several enzymes rather show decreased specific activities. In particular,

Fig. 1 Lamp-1 protein expression in normal human skin and in cultured human keratinocytes. Frozen section of the skin was labeled by indirect immunofluorescence (FITC) using the H4A3 antibody to human Lamp-1 and observed by fluorescence microscopy (**a**; magnification as in **b**) and by differential interference contrast microscopy (**b**; *bar* 100 μm). The expression of Lamp-1 is particularly demonstrated in keratinocytes as illustrated in the basal layer (insert of **a**; *arrow*). Lamp-1 protein is visualized by immunofluorescence in subconfluent keratinocyte culture (**c**; *bar* 40 μm)

the cathepsins B, C and L exhibit relatively low specific activities in post-confluent keratinocytes (Table 1).

Table 1 Enzymatic activity of lysosomal hydrolases in cultured human keratinocytes induced to differentiate in autonomous growth conditions

Enzyme	Ratio ± SD of specific enzymatic activity	
	In confluent/subconfluent keratinocytes[a]	In post-confluent/subconfluent keratinocytes[a]
α-Glucosidase	3.57 ± 0.70	3.53 ± 0.14
α-Mannosidase	2.01 ± 0.30	2.12 ± 0.11
α-Fucosidase	1.85 ± 0.18	1.50 ± 0.05
Hexosaminidase	1.34 ± 0.06	1.18 ± 0.08
β-Galactosidase	1.38 ± 0.06	0.90 ± 0.05
β-Glucoronidase	1.63 ± 0.11	1.59 ± 0.11
β-Glucosidase	2.25 ± 0.50	2.09 ± 0.91
Cathepsin B	1.11 ± 0.12	0.85 ± 0.00
Cathepsin C	1.29 ± 0.03	0.33 ± 0.02
Cathepsin L	0.79 ± 0.09	0.82 ± 0.08
Acid phosphatase	1.36 ± 0.08	1.08 ± 0.03

[a]The specific enzymatic activity (total enzymatic activity divided by the amount of cellular protein) of each lysosomal hydrolase was measured as described in Materials and methods in duplicate or triplicate autonomous cultures of human epidermal keratinocytes induced to differentiate by extension of the culture to confluence or post-confluence. This activity was then divided by the same specific enzymatic activity measured in autonomous cultures ($n = 6$) of non-differentiating keratinocytes at subconfluence

Culture confluence induces transient upregulation of Lamp-1 in epidermal keratinocytes

It has been demonstrated that cell confluence in autocrine cultures of keratinocytes controls the initiation of epidermal differentiation, including the expression of suprabasal K1 and K10 [35, 36, 43]. We tested both at protein and mRNA levels whether Lamp-1 expression varied when epidermal keratinocytes were grown to confluence and committed to differentiation. Indeed,
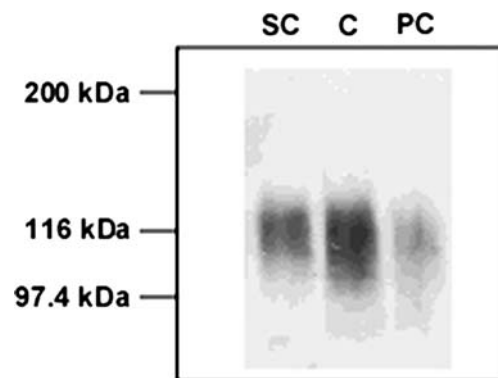

Fig. 2 Confluence of cultured keratinocytes transiently upregulates Lamp-1 protein expression. Proteins from subconfluent (*SC*), confluent (*C*) and post-confluent (*PC*) cultures of keratinocytes were extracted and identical amounts of proteins (13 μg) were analyzed by Western blotting with an antibody to Lamp-1. Positions of molecular weight markers (expressed in kDa) are indicated on the left

Western blot (Fig. 2) reveals a transient upregulation of Lamp-1 when the keratinocyte cultures reach cell confluence. Four days later, post-confluent cells show a decreased expression of Lamp-1 when compared to confluent and subconfluent cells (Fig. 2). The apparent molecular weight of Lamp-1 on SDS-PAGE is reduced in the post-confluent keratinocytes, suggesting partial degradation, probably deglycosylation of the protein.

At the mRNA level, culture confluence induces an elevated Lamp-1 mRNA expression, concomitant with the induction of the suprabasal K1 and K10, and with the increase in involucrin mRNA expression (Fig. 3). In post-confluent cultures, the amount of Lamp-1 mRNA is decreased (Fig. 3) in accordance with the detection of the protein (Fig. 2). This decrease in Lamp-1 expression at high cell density correlates with the suppressed expression of K1 and K10, whereas involucrin expression remains relatively high. This suggests that Lamp-1 expression can be modulated during the differentiation of epidermal keratinocytes and particularly upregulated in cultured keratinocytes at confluence.

This transient upregulation of Lamp-1 in the culture model seemed intriguing and we wondered whether there could be a particular reason for this regulation.
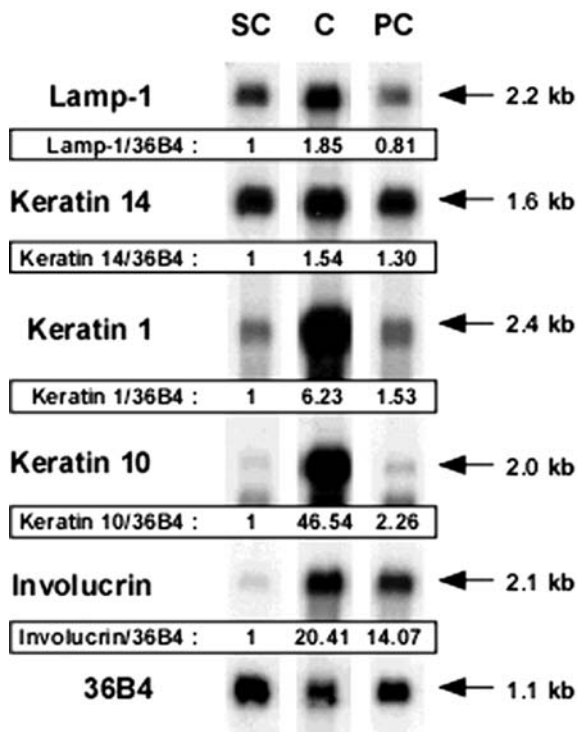

Fig. 3 Lamp-1 mRNA expression is transiently upregulated in confluent differentiating keratinocytes. Poly(A)RNA from subconfluent (*SC*), confluent (*C*) and post-confluent (*PC*) cultures of keratinocytes were extracted and analyzed by Northern blot hybridization. Two micrograms of each sample were used in each lane. Hybridization was performed with probes specific for Lamp-1, K14, K1, K10, involucrin and 36B4 transcripts. Ratios to 36B4 were determined by quantitative densitometry using the NIH image software program and normalized to levels in subconfluent (*SC*) cultures

Because of the possible interaction between Lamp-1 and galectins [41], we studied the expression of members of this family of animal lectins known to be expressed in the epidermis.

Cell density differentially regulates galectin expression in autocrine keratinocyte cultures

Northern blot analysis reveals that galectin-1 transcripts are only detected in subconfluent proliferating keratinocytes (Fig. 4). Indeed, following the onset of terminal differentiation at culture confluence, the gene expression of galectin-1 is apparently abrogated. Conversely, the gene expression of galectin-3 and galectin-7 is already detectable in subconfluent cultures of proliferating keratinocytes, but the expression of galectin-7 is notably upregulated as soon as keratinocytes are committed to differentiate at culture confluence (Fig. 4). Simultaneously, the galectin-3 expression is also regulated with epidermal differentiation and reaches its highest level in post-confluent cultures (Fig. 4), concomitantly with the expression of later markers of epidermal differentiation. These results show that galectins-1, -3 and -7 are expressed by epidermal keratinocytes and that the expression of their transcripts is regulated with the typical phenotypic variations demonstrated when the density of this cell type increases in culture.

The localization of galectin-3 detected by immunolabelling and confocal microscopy suggests that galectin-3 is already present in subconfluent keratinocytes and remains well expressed in post-confluent cells in both basal or suprabasal position in the culture (Fig. 5). Galectin-3 is partly found in keratinocytes in a position, which may correspond to the nucleus, an observation compatible with the splicing activity of this protein [6] and with its normal pattern in cells able to differentiate [50]. Lamp-1 does not particularly colocalize with galectin-3 in any culture condition (Fig. 5).

Discussion

In the present study, we show expression of Lamp-1 in the normal human epidermis and in normal cultured keratinocytes. In the epidermis, lysosomes of keratinocytes revealed by staining of Lamp-1 are mainly granules observed in the granular layer, maybe the lamellar granules involved in the keratinization and desquamation processes [14, 18]. Lysosomes in cultured keratinocytes seem abundant as soon as in subconfluent conditions, especially in comparison with cultured fibroblasts [16].

Looking at Lamp-1 during the differentiation of keratinocytes, we identify upregulation of Lamp-1 and upregulation of the specific activities of several lysosomal enzymes, particularly at the onset of differentiation concomitant with confluence of autocrine-cultured

Fig. 4 Galectin-1, -3 and -7 mRNA expressions are differentially regulated in autocrine keratinocyte cultures. **a** Expression of galectins mRNA in keratinocytes cultured at different cell density. Keratinocytes were grown in autocrine conditions and poly(A)RNA were extracted from subconfluent, confluent and post-confluent cultures. Then poly(A)RNA were analyzed using Northern blotting and hybridization with probes specific to galectin-1, -3 or -7. Exposures of each hybridized membrane were performed with screens fitting the PhosphorImager apparatus (Perkin-Elmer). The duration of the exposure was identical for each culture condition. Hybridization with the 36B4 probe was done in order to estimate gel loading. **b** Densitometric analysis of galectins expression. The results are presented as relative expression levels of galectins-1, -3 and -7 calculated as ratios to the expression of the 36B4 house-keeping gene
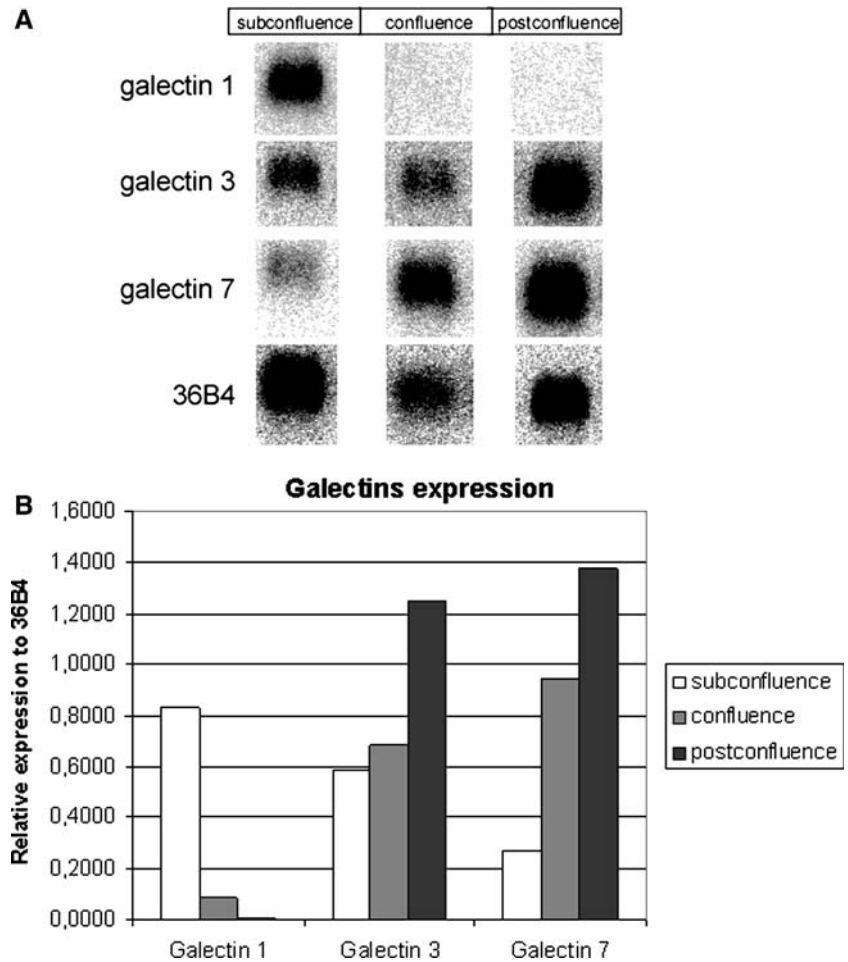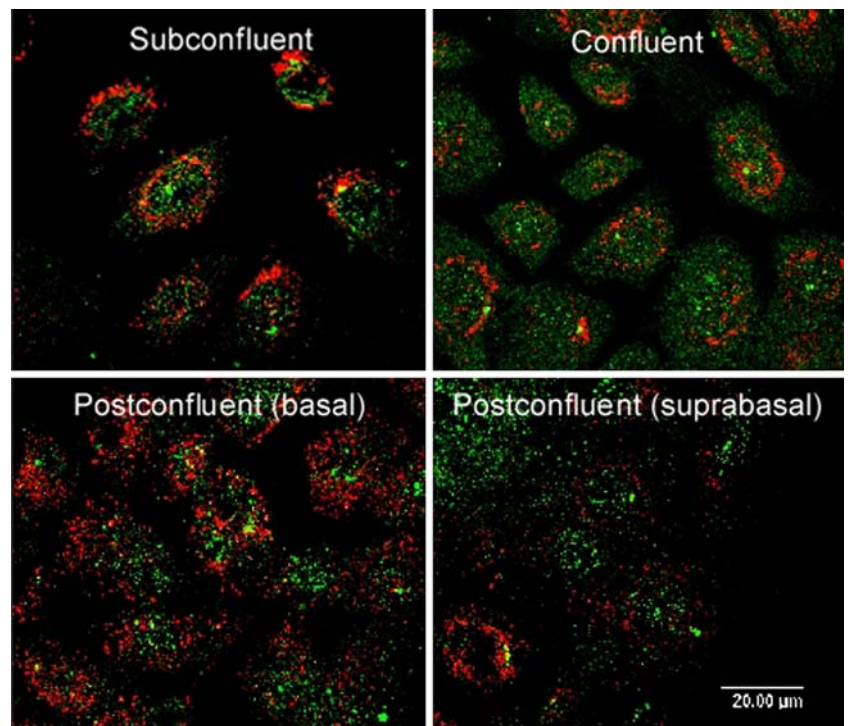

Fig. 5 Localization of galectin-3 and Lamp-1 proteins in autocrine cultures of keratinocytes. Keratinocytes of autocrine cultures were fixed when subconfluent, confluent, or post-confluent and analyzed after immunofluorescent labelling for galectin-3 (*green*) and Lamp-1 (*red*) using a Leica confocal microscope. In each culture condition, galectin-3 is distributed in the cytoplasm, and also in the nucleus, although Lamp-1 is found mainly in the cytoplasm, mostly with perinuclear localization. Galectin-3 and Lamp-1 are not predominantly co-localized in any culture condition

keratinocytes [35, 36]. Other studies of several cell types in culture have already suggested that lysosomes play a role in cells undergoing differentiation. For instance, in the intestinal cell lines HT-29 or Caco-2, the activity levels of lysosomal proteinases [9] and glycosidases [57] have been shown elevated in differentiating cells, compared with proliferating cells. Increased mRNA levels and enzymatic activities of certain lysosomal acid hydrolases were also observed in differentiated myocytes [44]. Only a few studies examined the activity of lysosomal hydrolases in human keratinocytes. Cathepsin B and L activities were found increased in differentiated rat-cultured keratinocytes [45]. Cathepsin L was shown to be involved in keratinocyte and melanocyte differentiation during mice hair follicle morphogenesis [46]. Our results reveal increased specific activities of lysosomal hydrolases upon differentiation. However, the decreased activities in post-confluent cultures could suggest that, for instance, some release of lysosomal proteins may occur after the triggering of differentiation. Such a release is proposed to contribute to desquamation at the surface of the epidermis [21]. Interestingly, we have recently demonstrated that such a lysosomal exocytosis can be induced by an intracellular elevation of calcium [23], a phenomenon accompanying the differentiation of epidermal cells and concomitant with a localization of Lamp-1 and -2 at the cell surface [23].

In the present study, the expression of Lamp-1 is transiently elevated at the onset of epidermal differentiation, when their clonogenic potential of keratinocytes is drastically suppressed [35, 54], and K1/K10 expression is upregulated [35]. However, the Lamp-1 expression is decreased 96 h later in post-confluent cultures containing keratinocytes expressing later markers of differentiation [36]. A similar concomitant elevation of Lamp-1 with cellular differentiation has been noticed in HC11 mammary epithelial cells [5]. Interestingly, only minor effects on Lamp-1 mRNA expression were observed in that study, suggesting a post-transcriptional control of Lamp-1 expression in HC11 cells [5]. A possible explanation for the discrepancy we noticed when comparing levels of Lamp-1 protein and transcript in our cultures of keratinocytes could be that Lamp-1 is partly degraded and deglycosylated in post-confluent keratinocytes. Indeed, Lamp-1 shows a reduced apparent molecular weight on Western blots in the post-confluent state (Fig. 3), a change also observed in differentiating Caco-2 intestinal cells [56]. Additionally, in both Caco-2 cells and keratinocytes, there is a clear increase in lysosomal enzyme activities with differentiation [57]. The specific activities of most lysosomal enzymes did not increase in post-confluent keratinocytes and those of cathepsins remained very low or even decreased in comparison with subconfluent cultures. Perhaps, it may be relevant to notice that endogenous inhibitors of cathepsins were reported as induced by differentiation in keratinocytes [45]. Moreover, the regulation of expression of a highly glycosylated Lamp-1 with epidermal differentiation is reminiscent of much older studies in which, by the use of

labelled lectins, alterations in the carbohydrate content of differentiating keratinocytes had been reported [3, 4].

This regulation of Lamp-1 in keratinocytes focused our interest towards testing epidermal galectins in keratinocytes, knowing for instance that galectin-7 is found especially in the epidermis [29]. Our results indicate that galectin-7 and -3 are upregulated with keratinocyte differentiation in culture, simultaneously with Lamp-1. It has been suggested that changes in galectin-3 level might be associated with differentiation of monocytes into macrophages, and with the cellular response to altered environmental conditions [10]. Our previous studies indicate that Lamps on the surface of some tumour cells serve as ligands for galectin-3 [41]. Based on that finding, we propose that the concomitant increase of Lamp-1 and galectins-3 and -7 with keratinocyte confluence may reflect a possible involvement in cell–cell interactions and perhaps in the epidermal stratification. Although galectin-3 is considered as a multifunctional protein, it still retains its unique role as a mediator of cell-to-matrix adhesion [26]. Epithelial cells lacking galectin-3 interact poorly with their extracellular matrices [31]. On the other hand, galectin-3 binds to cytokeratins, which might serve as cytoplasmic anchors regulating the transport and function of galectins in MCF7 and other cells [17]. It is not excluded that in differentiating keratinocytes, galectin-3, which is involved in protein sorting [8], may interplay with Lamp-1, perhaps on the cell surface, in order to direct the secretion of lamellar bodies [40]. Recently, it was reported that cultured keratinocytes at confluence do not require complementary signals for the onset of terminal differentiation except those given by cell–cell contact [25], but the localization of galectin-3 in cellular structures different than the ones where Lamp-1 is found denies any special interaction between the two proteins in those culture conditions.

Our results also show that the expression of galectin-1 is sharply regulated by cell density in autocrine cultures of keratinocytes. Galectin-1 transcripts were indeed detected in subconfluent highly proliferating keratinocytes only. Galectin-1 might thus play a role during keratinocyte proliferation.

Finally, one may wonder why keratinocytes exhibit so many lysosomes in the proliferating state. One possibility is that cells committed to differentiate in the epidermis must degrade adhesion molecules of the integrin family found exclusively in the basal layer [19]. This degradation is performed by integrin internalization and processing in lysosomes [20].

In summary, our results reveal that lysosomes are regulated by differentiation in human keratinocytes, resulting in increased activity of lysosomal hydrolases and Lamp-1 expression in confluent cultures. Expressions of galectins-1, -3 and -7 are differentially regulated in the same conditions. However, although Lamp-1 might be implied in the normal epidermal differentiation in vitro, it does not co-localize with galectin-3.

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