Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 11329–11334, September 1999
Cell Biology

Galectin-7 overexpression is associated with the apoptotic process in UVB-induced sunburn keratinocytes

(skin/apoptosis/cutaneous tumors)

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Communicated by Philip Hanawalt, Stanford University, Stanford, CA, July 23, 1999 (received for review March 8, 1999)

ABSTRACT **Galectin-7 is a β-galactoside binding protein specifically expressed in stratified epithelia and notably in epidermis, but barely detectable in epidermal tumors and absent from squamous carcinoma cell lines. Galectin-7 gene is an early transcriptional target of the tumor suppressor protein P53 [Polyak, K., Xia, Y., Zweier, J., Kinzler, K. & Vogelstein, B. (1997) *Nature (London)* 389, 300–305]. Because p53 transcriptional activity is increased by genotoxic stresses we have examined the possible effects of ultraviolet radiations (UVB) on galectin-7 expression in epidermal keratinocytes. The amounts of galectin-7 mRNA and protein are increased rapidly after UVB irradiation of epidermal keratinocytes. The increase of galectin-7 is parallel to P53 stabilization. UVB irradiation of skin reconstructed *in vitro* and of human skin *ex vivo* demonstrates that galectin-7 overexpression is associated with sunburn/apoptotic keratinocytes. Transfection of a galectin-7 expression vector results in a significant increase in terminal deoxynucleotidyltransferase-mediated UTP end labeling-positive keratinocytes. The present findings demonstrate a keratinocyte-specific protein involved in the UV-induced apoptosis, an essential process in the maintenance of epidermal homeostasis.**

The epidermal layer of skin is a stratified and keratinized epithelium whose major function is to protect our body against environmental stresses. Two cell compartments with distinctive proliferating capacities can be distinguished in epidermis: the basal cell layer that contains multiplying keratinocytes and the suprabasal compartment that contains keratinocytes that have ceased to divide and undergo differentiation (1). Basal and suprabasal cells also can be distinguished on the basis of morphological features and expression of specific biochemical markers. For instance, basal keratinocytes express the K5/K14 keratin pair (2) and specific integrins (3). Suprabasal keratinocytes express the K1/K10 keratins pair instead of the K5/K14 pair (2). Synthesis of cell envelope precursors and other terminal differentiation proteins (4) are signs of the ultimate phases of keratinocyte differentiation, which culminate with the assembly of cornified layers.

The permanent renewal of epidermis and its ability to brave accidental situations as well (e.g., burn and cuts) hints at the presence of stem cells and their rapidly proliferating progeny (transient amplifying cells) (5). It also requires the rapid removal of cells damaged irreparably after exposure to environmental stresses (e.g., after UV irradiation) and condemned to death through the apoptotic process (6). The balance between proliferation, stratification, differentiation, and apoptosis therefore is essential to maintain epidermal homeostasis.

In an attempt to isolate new markers of the normal process of epidermal differentiation we previously have cloned a cDNA encoding a novel β-galactoside-binding protein (7), galectin-7 (8, 9). Galectin-7 is the first marker of epithelial stratification whose expression does not depend on local differentiation: it is expressed in all cell layers in epidermis and also in the cornea and oesophagus although the latter and former tissues express distinctive sets of differentiation markers such as specific keratins (2, 9). In contrast, galectin-7 is not expressed in simple epithelia from endodermal origin such as the kidney or the liver (9, 10). We have demonstrated that the onset of galectin-7 expression coincides with the onset of stratification in the developing mouse embryo (10). Several lines of evidence have supported the idea that an inverse relation may exist between galectin-7 expression and keratinocyte proliferation. First, galectin-7 expression was found abrogated in cell lines derived from epidermal tumors called squamous cell carcinomas (SCCs) (9) and also in keratinocytes transformed by the large T antigen (LT) of simian virus 40 (i.e., where P53 tumor suppressor protein is sequestered by LT) (ref. 11; see *Results*). Second, galectin-7 is barely detectable in human basal cell carcinomas and SCCs and in SCCs induced in the mouse (9). Third, high level of galectin-7 mRNAs was detected mostly in the suprabasal (postmitotic) epidermal compartment of the developing mouse embryo whereas high level of galectin-7 mRNAs was detected in both basal and suprabasal compartment of adult epidermis (which both are poorly proliferating) (10). In addition, the galectin-7 gene was found among the 14 early-response genes induced ectopically in a colorectal carcinoma cell line during P53-induced apoptosis (12).

In the present study we refined the characterization of galectin-7 by using classical cultures of human epidermal keratinocytes, a model of human skin reconstructed *in vitro* (13), and human epidermis. We examined the effects of UV wavelengths (UVB) and doses known to result in stabilization of the P53 protein (14–17). We found that the amount of galectin-7 mRNA and protein was increased significantly in cultured keratinocytes after UVB irradiations. In addition, using human skin reconstructed *in vitro* and human skin irradiated *ex vivo*, we demonstrated that UVB-induced galectin-7 overexpression mostly occurs in sunburn cells, i.e. corresponding to apoptotic keratinocytes (18–20). Furthermore galectin-7 overexpression after transfection of an expression vector was accompanied by a significant increase in terminal deoxynucleotidyltransferase-mediated UTP end labeling (TUNEL)-positive keratinocytes. Our findings show that galectin-7 participates to the process of UVB-induced apoptosis in epidermis.

METHODS

Tissue Culture. *Skin samples, keratinocyte, and fibroblast cultures.* Normal human skin was obtained from plastic mam-

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Abbreviations: TUNEL, terminal deoxynucleotidyltransferase-mediated UTP end labeling; SCC, squamous cell carcinoma; LT, large T antigen; CMV, cytomegalovirus; GFP, green fluorescent protein.

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mary reduction. Human normal epidermal keratinocytes and SCC13 keratinocytes (21) were obtained and cultured as described (22) on a feeder layer of lethally irradiated Swiss 3T3 fibroblasts. Human dermal fibroblasts isolated after spreading from mammary skin explants and simian virus 40 LT-transformed SVK14 keratinocytes (23) were cultured in DMEM containing 10% FCS. SCC13 keraninocytes bear a point mutation in the *p53* gene in exon 7, nucleotide 772 (G to A), leading to a Glu to Lys amino acid change at position 258 (DNA binding domain) (24).

Skin samples. Pieces of human skin isolated from fresh samples as described above were maintained in culture at the air-liquid interface (*ex vivo*) during 24 hr as performed for reconstructed skin (see below).

Reconstructed skin in vitro. Reconstruction of differentiated epidermis on the dermal equivalents was performed at the air-liquid interface culture condition as described (25).

Irradiation Source and Procedure. Growth and UVB irradiations of classical cultures of epidermal cells (i.e., grown on plastic) were performed as described (26). UVB irradiations of skin samples *ex vivo* or of skin reconstructed *in vitro* were performed as described (20). Briefly, UVB irradiations were performed by using Philips (Lumière Service, Paris) TL20W/12 fluorescent tubes. The wavelengths shorter than 290 nm were eliminated by using a Kodacel (Kodak) filter. The wavelength spectrum was carefully checked with a Macam (Edinborough, Scotland) SR3010 spectroradiometer. The irradiance measured by using an Osram (Berlin) Centra dosimeter was 0.250 mW/cm^2 at 20 cm from the source. Before UVB exposure, keratinocyte culture medium was replaced by PBS (BioMerieux, Charbonnier les Bains, France). Normal human skin or reconstructed skin samples on grids were transferred into new dishes and irradiated without medium.

RNA Purification and Northern Blotting. Total RNAs were prepared according to the method of Chomczynski and Sacci (27) and analyzed (10 μg/lane) by Northern blot as described in detail (8). Quantification of signals were obtained after scanning the blots on a FluorImager (Molecular Dynamics) with IMAGEQUANT software (Molecular Dynamics).

Protein Extraction and Western Blotting. Proteins were prepared and processed for Western analysis (20 μg/lane) as described (28). The α-Gal-7.2 rabbit antiserum (9) was used diluted at 1/350. The anti-P53 mAb (DO-7, Immunotech, Luminy, France) was used diluted at 1/500.

Histology. Samples were fixed in 10% neutral formalin and processed for histology. Paraffin sections were stained with heamatoxylin, eosin, and saffron.

Immunostainings. *Antibodies.* Rabbit polyclonal antiserum directed against human galectin-7 (α-Gal-7.2 antiserum) was obtained as described (9) and used diluted (1/100) in PBS. FITC-conjugate or rhodamine-conjugate swine anti-rabbit immunoglobulins (Dako) were used at 1/100 as secondary antibodies.

Procedure. Immunolabelings were performed on air-dried 5-μm vertical cryosections as described (20).

TUNEL Method. TUNEL reaction was carried out by using the *In Situ* Cell Death Detection Kit (Boehringer Mannheim) on 4% formaldehyde fixed frozen sections as described in detail (20).

TUNEL Combined with Anti-Galectin-7 Immunostaining. The procedure was carried out as described (29) on 1% paraformaldehyde-fixed sections. Incubation in TUNEL reaction was performed at +37°C for 25 min. Sections were incubated in 5% normal goat serum before immunolabeling procedure.

Plasmids and Cell Transfections. The galectin-7 cDNA insert (1A12, ref. 8) was released from pSKII+ vector (Stratagene) by *Hin*dIII/*Xba*I enzymatic digestion and then cloned under the control of the cytomegalovirus (CMV) promoter in the cohesively *Nhe*I/*Hin*dIII digested expression vector pBK-CVM (Stratagene). Fifty percent confluent SCC13 keratinocytes were transfected in duplicate by the polybrene/DMSO chock technique (30) using 0.36 μg/cm^2 of either pBK-CMV or pEGFP-N1 (CLONTECH) as controls, or pBK-CMV-1A12. Eighteen, 36, or 54 hr after transfection cells were fixed and processed for TUNEL combined with galectin-7 immunostaining. Cells in culture supernatant were cytocentrifuged as described (28) and processed for labeling and TUNEL.

RESULTS

Galectin-7 mRNA and Protein Are Increased in Human Epidermal Keratinocytes Exposed to UVB Radiations. Normal human keratinocytes were kept 6 days in culture after confluency and UVB-irradiated. The amount of galectin-7 mRNA analyzed by Northern blot increases significantly as early as 1 hr after UVB exposure (500 J/m^2) with a maximum at 3–4 hr. This increase lasts about 8 hr. A normal level of galectin-7 mRNA is recovered by 24 hr after UVB irradiation and then is decreased below the initial level at 48 hr. Quantitation of the relative amount of galectin-7 mRNA to that of glyceraldehyde-3-phosphate dehydrogenase mRNA indicates that 3–4 hr after exposure of cells to UVB irradiation, an average 2.7 times more galectin-7 mRNA is found as compared with the unirradiated sample (Fig. 1*A* and *B*). Twenty-four and 48 hours after irradiation the relative level of galectin-7 mRNA decreases below the initial value, a phenomenon likely resulting from elimination of apoptotic keratinocytes that overexpress galectin-7 (see Fig. 5 and Table 1).

The amount of galectin-7 protein was estimated by Western blots using protein extracts prepared from epidermal keratinocytes harvested 24 and 48 hr after UVB irradiation and a rabbit polyclonal antiserum raised against the carboxyl-terminal end of the protein (α-Gal-7.2; ref. 9). Fig. 1*C* shows that the amount of galectin-7 protein increases significantly 24 hr after UVB irradiation. This increase is sustained for 48 hr after UVB treatments (400 and 800 J/m^2). A second hybridization of the same Western membrane with a specific mAb (DO-7, see *Methods*) reveals that the P53 protein is stabilized after exposure to UVB. The stabilization of P53 parallels the increase of galectin-7 protein (Fig. 1*C*). Galectin-7 mRNA and proteins are neither detected in SCC13 cells (21) (data not shown) nor in a keratinocyte cell line (SVK14) transformed by the simian virus 40 LT (11, 23) (Fig. 1*C*). UVB irradiation of both cell lines does not induce expression of galectin-7 at a detectable level. This is illustrated by using protein extracts from SVK14 keratinocytes in Fig. 1*C*.

UVB Irradiation Induces High Level of Galectin-7 Expression in Apoptotic Keratinocytes. *Normal human skin ex vivo*. Freshly biopsied normal human skin was either sham- or UVB-irradiated (750 J/m^2), incubated for 24 hr at the air-liquid interface, and then processed for routine histology, TUNEL reaction, or galectin-7 immunostaining (Fig. 2). Histological observations of the irradiated sample reveals that UVB exposure induces the presence of numerous sunburn cells within the suprabasal compartment of the epidermis (Fig. 2 *A* and *B*). These cells display the typical eosinophylic cytoplasm and the condensed nucleus as described (18). Detection of DNA fragmentation using the TUNEL assay (Fig. 2 *C* and *D*) shows that positive nuclei are restricted to the uppermost layers of the viable epidermis in the sham-irradiated sample (Fig. 2*C*) as expected normally in late stages of epidermal differentiation (31). In UVB-exposed samples (Fig. 2*D*), numerous TUNEL-positive nuclei corresponding to apoptotic keratinocytes are observed in the suprabasal layers of the epidermis. The galectin-7 immunostaining (Fig. 2 *E* and *F*) confirms that all epidermal layers are fluorescent in the non-UVB exposed skin (Fig. 2*E*), as observed previously (9). Twenty-four hours after irradiation, the galectin-7 labeling also is observed throughout the depth of viable epidermis, but importantly, some suprabasal keratinocytes that could be apoptotic display a significantly higher galectin-7 fluorescence.

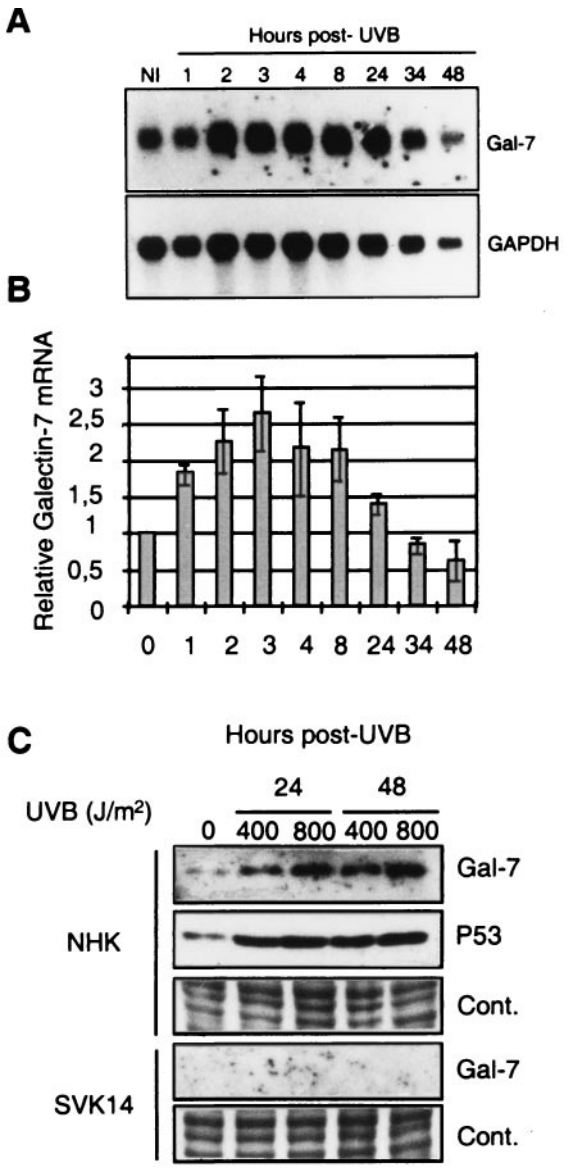

FIG. 1. UVB irradiation increases the amount of galectin-7 mRNA and proteins. (*A*) Normal human epidermal keratinocytes were UVB-irradiated (500 J/m^2) and incubated for the indicated times (hours post-UVB) before RNA extraction and Northern blotting. A significant increase of galectin-7 mRNA relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA is observed after UVB irradiation. (*B*) Scanning quantitation of blots after normalization of galectin-7 values to those of GAPDH. The average relative increase (×2.7) of the galectin-7 mRNA occurs 3–4 hr after irradiation and decreases below the initial value 48 hr after UVB irradiation. (*C*) Normal human epidermal keratinocytes (NHK) and SVK14 keratinocytes (SVK14) were UVB-irradiated at the doses indicated (400 or 800 J/m^2) and incubated for 24 and 48 hr as indicated. Proteins then were prepared and analyzed by Western blot. Both UVB doses result in the increase of galectin-7 and P53 in NHK. These increases were more important after 800 J/m^2 UVB. In SVK14 keratinocytes, galectin-7 was neither detected before or after UVB irradiation. Cont. shows a loading control (i. e. Coomassie blue staning of a portion of the gel) after transfer.

Skin reconstructed in vitro. Skin reconstructed *in vitro* was either sham- or UVB-irradiated (700 J/m^2) and harvested for analysis 24 hr after irradiation (Fig. 3). Histological analysis of sham-irradiated skin demonstrates that the reconstructed epidermis displays a complete differentiation comprising granular and horny layers as described (20) (Fig. 3*A*). UVB irradiation induces an alteration of epidermal differentiation including a decrease of the granular layer and the formation of typical (i.e., eosinophilic) sunburn cells in the suprabasal layers (Fig. 3*B*). Alike in human skin, in sham-irradiated skin reconstructed *in vitro*, rare positive nuclei located just underneath the horny layer are revealed by the TUNEL reaction (Fig. 3*C*). In UVB-irradiated skin reconstructed *in vitro*, numerous apoptotic keratinocytes are revealed by the TUNEL reaction within the suprabasal compartment of epidermis (Fig. 3*D*). In control sham-irradiated samples, immunolabeling using the anti-galectin-7 antibody reveals galectin-7 expression in all living epidermal layers (Fig. 3*E*). In UVB-irradiated reconstructed skin, numerous cells within the suprabasal compartment of epidermis display much a higher level of galectin-7 expression than in other keratinocytes (Fig. 3*F*).

Table 1. Transfection of a galectin-7 expression vector increases TUNEL-positive nuclei in SCC13 cells

	Hours posttransfection		
	18	36	54
TUNEL	5.7	11.1	3.3
Floating cells	1.7	2.2	5.7

SCC13 keratinocytes grown on slides and floating cytocentrifuged cells were processed for immunolabeling of galectin-7 and TUNEL reactions. TUNEL indicates the ratio of (TUNEL-positive/total) cells in pBK-CMV-1A12-transfected cells to (TUNEL-positive/total) cells in pBK-CMV-transfected cultures. Floating cells indicates the ratio of floating cells in pBK-CMV-1A12- to pBK-CMV-transfected cultures. Results are from mean cell numbers (+/− 10%) obtained in two independent experiments.

Keratinocytes Expressing High Levels of Galectin-7 Are Apoptotic. Skins reconstructed *in vitro* were irradiated with 700 J/m^2 UVB and processed after either 8 (Fig. 4 *A–C*) or 24 hr (Fig. 4 *D–F*). To visualize on the same section the galectin-7 protein and the apoptotic nuclei, samples were labeled by using a method combining immunostaining and TUNEL techniques (see *Methods*). Double labeling of irradiated samples reveals that some cells (indicated by arrows in Fig. 4 *A–D*) clearly display coincidental high level of galectin-7 expression and TUNEL stainings. The intensity of the galectin-7 immunostaining is lower at 8 hr (Fig. 4 *A–C*) than at 24 hr (Fig. 4 *D–F*) after irradiation. Twenty-four hours after irradiation, the presence of numerous apoptotic bodies caused by the important DNA fragmentation makes the vizualization of complete individual cells difficult. The colocalization of DNA fragmentation and high level of galectin-7 stainings led to the conclusion that galectin-7 is overexpressed in apoptotic keratinocytes in epidermis of UVB-irradiated reconstructed skin.

Galectin-7 Overexpression Induces TUNEL-Positive Keratinocytes. SCC13 cells that do not express detectable level of galectin-7 (Fig. 5*A* and ref. 9) were mock-transfected by using the control plasmid pBK-CMV (Fig. 5 *C* and *D*), or transfected by using plasmids encoding either the green fluorescent protein EGFP (pEGFP-N1) (Fig. 5 *E–G*) or galectin-7 (pBK-CMV-1A12) (Fig. 5 *H* and *I*). In nontransfected cells the number of TUNEL-positive cells was insignificant (Fig. 5*B*). In all transfection conditions (C–I), the polybrene/DMSO transfection technique results in a slight increase of attached TUNEL-positive cells (Fig. 5 *D*, *G*, and *I*) as compared with nontransfected cells (Fig. 5 *B*). This effect results probably from DNA damage and breaks induced by DMSO (32). Transfection of the galectin-7 expression vector results in galectin-7 overexpression (Fig. 5*H*) and in a maximum 11-fold increase of TUNEL-positive cells 36 hr after transfection (Fig. 5*I*) as compared with control plates (i.e. mock-transfected) (Fig. 5*D*, Table 1), or transfected by using pEGFP-N1 (Fig. 5*G*). The number of cells floating in culture supernatant is increased 5.7-fold 54 hr after transfection of the galectin-7 expression vector (Table 1), suggesting that galectin-7 overexpression may promote cell detachment. These observations

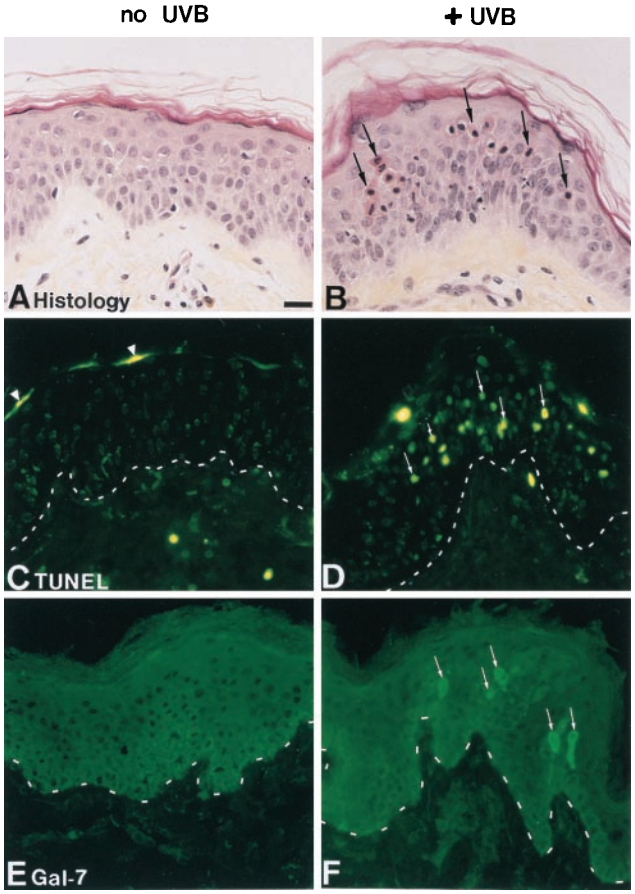

FIG. 2. Galectin-7 expression is increased in sunburn keratinocytes formed in normal human skin *ex vivo* after UVB exposure. Normal human skin on grids was sham-irradiated (*A*, *C*, and *E*) or exposed to UVB 750 J/m^2 (*B*, *D*, and *F*). Twenty-four hours after irradiation, samples were processed for histology (*A* and *B*), TUNEL reaction (*C* and *D*), and galectin-7 immunostaining (*E* and *F*). Histological examination reveals that UVB irradiation induces the formation of numerous sunburn cells within the suprabasal compartment of the epidermis (*B*, arrows). In sham-irradiated skin, rare positive nuclei restricted to the terminally differentiated keratinocytes are detected by the TUNEL reaction (*C*, arrowheads). In UVB-irradiated samples, numerous apoptotic/TUNEL-positive keratinocytes are detected within the suprabasal epidermal compartment (*D*, arrows). In sham-irradiated samples, immunolabeling using the anti-galectin-7 antibody reveals that all living epidermal cell layers express galectin-7 (*E*). In UVB-irradiated samples, galectin-7 labeling also is observed throughout the epidermal compartment. In addition, UVB irradiation induces a much higher level of galectin-7 expression in some suprabasal keratinocytes (*F*, arrows). Gal-7 indicates sections labeled by using the anti-galectin-7 antiserum; TUNEL indicates sections processed for the TUNEL labeling. Dotted lines indicate the dermal-epidermal junction. (Bar: 25 μm.)

demonstrate that galectin-7 plays an active role in inducing the apoptotic process in epidermal keratinocytes.

DISCUSSION

The aim of the present study was to gain insight into the potential role of galectin-7 in epidermal homeostasis as previously suggested (9, 10). We took advantage of the recent discovery by Polyak and colleagues (12), showing that galectin-7 gene is among the 14 genes identified of 7,000 screened whose expression is induced during early steps of P53-dependent apoptosis. Because UVB radiations are efficient modulators of gene expression, including *p53* (14, 16) and induce apopotosis in epidermal keratinocytes (15, 16, 19, 20, 33–36) we assessed galectin-7 expression after UVB irradiation.

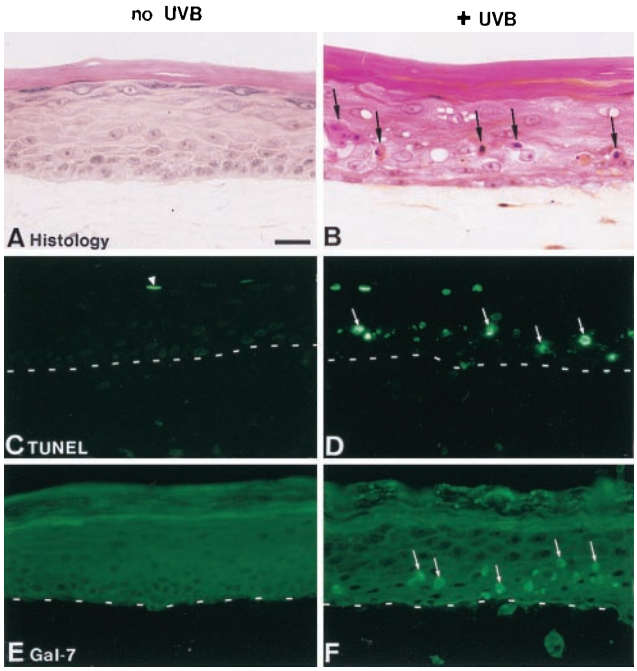

FIG. 3. Galectin-7 expression is increased in sunburn keratinocytes formed in skin reconstructed in vitro after UVB exposure. Sham-irradiated (*A*, *C*, and *E*) or UVB (750 J/m^2)-exposed samples (*B*, *D*, and *F*). Twenty-four hours after irradiation samples were processed for histology (*A* and *B*), TUNEL reaction (*C* and *D*), and galectin-7 immunostaining (*E* and *F*). The sham-irradiated skin reconstructed *in vitro* displays a complete differentiation as attested by the presence of granular and horny layers (*A*). UVB irradiation induces an important decrease of the granular layer and the formation of numerous typical sunburn cells (*B*, arrows). In sham-irradiated samples, rare TUNEL-positive nuclei are detected in the uppermost epidermal layers (*C*, arrowhead). In UVB-irradiated samples numerous apoptotic keratinocytes are in the suprabasal layers of the epidermal compartment (*D*, arrows). In sham-irradiated samples, galectin-7 is detected throughout all living epidermal layers (*E*). In UVB-irradiated samples, galectin-7 also is detected in all living epidermal layers. In addition, UVB irradiation induces a much higher level of galectin-7 expression in some suprabasal keratinocytes (*F*, arrows). Gal-7 indicates sections labeled by using the anti-galectin-7 antiserum; TUNEL indicates sections processed for the TUNEL labeling. Dotted lines indicate the junction between the epidermis and the dermal equivalent. (Bar: 25 μm.)

High Level of Galectin-7 Expression in Sunburn/Apoptotic Keratinocytes. The major finding of the present study is that sunburn/apoptotic keratinocytes express higher levels of galectin-7 protein than those of other keratinocytes. This observation suggests that galectin-7 is strongly associated with UVB-induced apoptosis in epidermis. Other galectins also affect cell fate decisions such as differentiation, proliferation, and apoptosis. For instance, galectin-1 is known to trigger the differentiation of muscle cells (37), but it also may be mitogenic in vascular cells (38), cytostatic in fibroblasts (39) or proapoptotic in T lymphocytes (ref. 40; for review see ref. 41). On the other hand, galectin-3 behaves as an antiapoptotic molecule that would act through its Bcl-2 like domain BH-1 (42). It also is involved in kidney tubulogenesis where it can modulate cell-cell and cell-substratum interactions (43). Inactivation of galectin-3 alleles in mouse also has put forward its role in chondrocyte differentiation (C. Colnot and F. Poirier, personal communication).

From the comparison of developmental expression of several galectins, including galectin-1, -3, and -7, it was worth noting, however, that galectin-7 transcripts have a remarkable and distinctive degree of tissue specificity (10, 44). Galectin-7 is the only member of this family whose expression is restricted

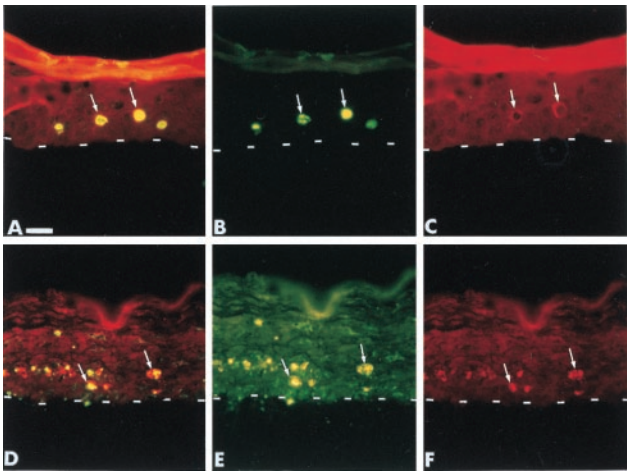

FIG. 4. UVB-induced galectin-7 overexpression occurs in TUNEL-positive apoptotic keratinocytes. Skins reconstructed *in vitro* were UVB-irradiated (700 J/m^2). Eight hours (*A–C*) or 24 hr (*D–F*) after irradiation, samples were labeled by using a combined method of TUNEL reaction (green) and galectin-7 immunostaining (red) on the same section. Double detection (*A* and *D*), TUNEL reaction (*B* and *E*), and galectin-7 immunostaining (*C* and *F*). Arrows indicate cells that display simultaneously a TUNEL-positive nucleus as well as a strong immunostaining for galectin-7. The intensity of the galectin-7 immunostaining is lower at 8 hr than at 24 hr after irradiation. Note the very important DNA fragmentation and the presence of numerous apoptotic bodies 24 hr after UVB irradiation. Dotted lines indicate the junction between epidermis and the dermal equivalent. (Bar: 25 μm.)

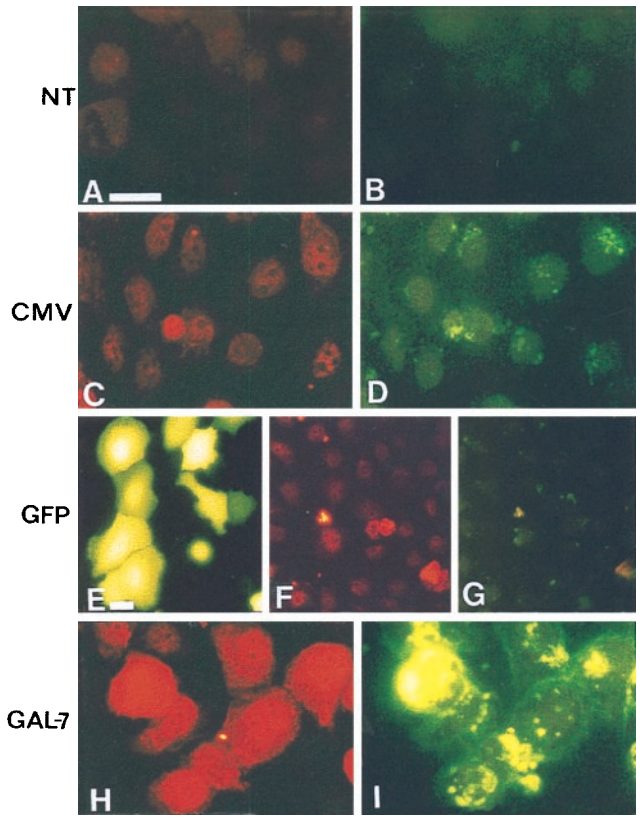

FIG. 5. Galectin-7 overexpression results in increase in TUNEL-positive keratinocytes. SCC13 keratinocytes were not transfected (NT) (*A* and *B*), mock-transfected with pBK-CMV (CMV) (*C* and *D*), transfected with the pCMV-EGFP (GFP) expression vector (*E–G*), or with the pBK-CMV-1A12 (GAL-7) expression vector (*H* and *I*) and processed for galectin-7 immunolabeling (*A*, *C*, *F*, and *H*) and TUNEL reaction (*B*, *D*, *G*, and *I*). (*E*) Cells transfected by using the pEGFP plasmid before processing for galectin-7/TUNEL labeling. As shown in this representative experiment photographed 36 hr after transfection, overexpression of galectin-7 (*H*) is accompanied by an important increase (×11, Table 1) of TUNEL-positive cells (*I*). Note that transfection conditions result in a slight increase of nuclei staining by using both the anti-galectin-7 antiserum and the TUNEL technique. (Bars: *A–D*, *H*, and *I*, 18 μm; *E–G*, 9 μm.)

to epithelia that are, or are destined to become stratified, especially the epidermis (9, 10).

The amount of galectin-7 mRNA and proteins was increased significantly in postconfluent (classical) cultures of epidermal keratinocytes exposed to UVB radiations. UVB doses were chosen to obtain 50% cell survival (i.e., 800 J/m^2) or more (400 J/m^2) as determined in our laboratory by using clonal analysis (26). It was worth noting that the average 2.7-times increase of galectin-7 mRNAs we measured after UVB irradiation was lower than the 30-times induction of galectin-7 mRNAs observed by Polyak and colleages (12) in colorectal carcinoma cells (DLD-1 cells) infected with a recombinant adenovirus expressing p53. This differential increase of galectin-7 mRNA amounts presumably is caused by the fact that DLD-1 cells do not normally express galectin-7 at a detectable level whereas normal epidermal keratinocytes do. It is also possible that transcriptional increase of galectin-7 mRNA after UVB occurs only in some cells as suggested by Figs. 2 and 3 and also may be hampered because of UV-induced photolesions. The increase in galectin-7 expression after UVB irradiation is reminiscent of other results showing that expression of lectins may be induced by UV radiations at the surface of a human carcinoma cell line (45). The increase in galectin-7 expression paralleled accumulation of P53, supporting the hypothesis that tumor suppressor protein P53 controls galectin-7 transcription (9, 12). In contrast, galectin-7 expression was not detected in standard culture conditions nor it was induced after UVB irradiation in human keratinocyte cell lines lacking wild-type P53 or where P53 is sequestered by the LT of the simian virus 40 (present study, Fig. 1*C*, and refs. 9 and 11). In addition, our preliminary experiments have indicated that galectin-7 is barely detectable in mice bearing inactivated *p53* alleles (I. Cail and F. Poirier, personal communication). Our current investigations should determine whether galectin-7 is modulated after exposure to UVs in those p53 knockout mice that display reduced capacity to UV-induced sunburn cell formation (19) and are prone to UV-induced SCC (46).

Using a three-dimensional model of skin reconstructed *in vitro* comprising a fully differentiated epidermis, we were able to demonstrate that UVB doses close to one biological efficient dose determined previously (i.e., 500 J/m^2, ref. 20) induced high levels of galectin-7 expression in keratinocytes exhibiting the morphological features of sunburn cells (18). Double-labeling of DNA fragmentation and galectin-7 expression demonstrated that high levels of galectin-7 expression did occur in sunburn/apoptotic keratinocytes as shortly as 8 hr after irradiation. Very importantly, overexpression of galectin-7 in sunburn keratinocytes also was observed in human skin *ex vivo* after UVB irradiation. The present findings put forward the role of galectin-7 in UVB-induced apoptosis of epidermal keratinocytes and further support previous studies (20, 47, 48) regarding the usefulness of skin reconstructed *in vitro* in evaluating effectors of cell fate and differentiation in epidermis.

Galectin-7 Seems to Play a Role in Epidermal Apoptosis. The apoptotic process is essential to maintain epidermal integrity. Loss of bona fide P53 functions may confer growth advantages and exacerbates cell susceptibily to genotoxic effects of UVs [i.e. the introduction in DNA of major damages such as cyclobutane pyrimidine dimers (CPDs) and 6–4 photoproducts], thus favoring the stepwise introduction of selectable mutations influencing growth control (49) (for review see ref. 50). However, up to 4% of cells present in normal-appearing, sun-exposed skin areas bear p53 mutations (49) whereas very few of them develop into precancerous (actinic keratosis) or

cancerous (SCC) lesions (51). Most keratinocytes harboring a mutated *p53* but that have retained the ability to undergo squamous differentiation may then escape (pre) neoplastic transformation (6, 51). This conclusion is strongly supported by the location of sunburn/apoptotic keratinocytes in the basal (proliferative) or parabasal layer of reconstructed epidermis at an early time (6 hr) after UVB irradiations whereas CPD DNA lesions are spread throughout the depth of (nucleated) epidermis (20). Sunburn cells then become suprabasal within 24 hr after irradiations and eventually are eliminated by 48 hr (20). In this respect, galectin-7 overexpression seems to play an active role in the process of keratinocyte apoptosis as attested by a significant increase of TUNEL-positive keratinocytes and floating cells in galectin-7-transfected plates. These observations indicate that cells located in the basal layer (i.e., that can give rise to basal carcinomas or SCCs; refs. 5 and 52) may escape neoplastic transformation by entering the apoptotic or the differentiation pathways. Galectin-7 overexpression might be essential to the apoptotic process in epidermis.

One aspect of altered or abrogated P53 expression is the absence of induction of its transcriptional targets such as the Bax "death gene" (53). We previously have demonstrated that several cell lines obtained from SCC (bearing mutant P53 alleles) or after transformation by the simian virus 40 LT that inactivates P53 functions do not express galectin-7 at a detectable level (Fig. 1*C*; ref. 9 and references therein). In addition, exposure to these cell lines toward UVB radiation does not induce galectin-7 (Fig. 1*C*). These observations suggest that UV-induced galectin-7 overexpression in sunburn/apoptotic keratinocytes occurs in cells that (*i*) bear wild-type P53 function, and (*ii*) are endowed with a proliferation potential because epidermal cells may embark squamous differentiation independently of *p53* integrity (54).

In light of previous studies demonstrating that galectin-1 modulates carbohydrate-dependent attachment of cells to the basement membrane (37, 55) and triggers the differentiation of muscle (37) it will be important to determine by immunolabeling whether galectin-7 is externalized, for instance at the basal pole of basal keratinocytes, and may modulate their attachment to the basement membrane. Secretion of galectin-7 in suprabasal keratinocytes also might affect intercellular adhesion and participate to their rapid elimination. UVB-induced overexpression of galectin-7 thus would (*i*) stimulate detachment of apoptotic keratinocytes in the basal epidermal layer, and (*ii*) contribute to their subsequent elimination upon travel across suprabasal layers. These hypotheses suggest that mutations in the galectin-7 gene might be associated with epidermal disorders. Clues to the role of galectin-7 also should be provided by the generation of mice bearing inactivating mutations of the galectin-7 gene.

We gratefully acknowledge Dr. Francoise Poirier and Isabelle Cail for privileged communications of unpublished data. We are also grateful to Miss Corinne Vious for her expert technical assistance. We are indebted to Drs. Jacques Leclaire and Daniel Asselineau for their continuous support and critical reading of the manuscript. We also thank Drs. Yann Barrandon and James Rheinwald for the gift of SCC13 cells. T.M. is supported by funds (ARC Grant 1711) from the Association pour la Recherche sur le Cancer.

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