Identification and Characterization of a Novel, Psoriasis Susceptibility-related Noncoding RNA gene, PRINS*

To identify genetic factors contributing to psoriasis susceptibility, gene expression profiles of uninvolved epidermis from psoriatic patients and epidermis from healthy individuals were compared. Besides already characterized genes, we identified a cDNA with yet unknown functions, which we further characterized and named PRINS (Psoriasis susceptibility-related RNA Gene Induced by Stress). In silico structural and homology studies suggested that PRINS may function as a noncoding RNA. PRINS harbors two Alu elements, it is transcribed by RNA polymerase II, and it is expressed at different levels in various human tissues. Real time reverse transcription-PCR analysis showed that PRINS was expressed higher in the uninvolved epidermis of psoriatic patients compared with both psoriatic lesional and healthy epidermis, suggesting a role for PRINS in psoriasis susceptibility. PRINS is regulated by the proliferation and differentiation state of keratinocytes. Treatment with T-lymphokines, known to precipitate psoriatic symptoms, decreased PRINS expression in the uninvolved psoriatic but not in healthy epidermis. Real time reverse transcription-PCR analysis showed that stress signals such as ultraviolet-B irradiation, viral infection (herpes simplex virus), and translational inhibition increased the RNA level of PRINS. Gene-specific silencing of PRINS by RNA interference revealed that down-regulation of PRINS impairs cell viability after serum starvation but not under normal serum conditions. Our findings suggest that PRINS functions as a noncoding regulatory RNA, playing a protective role in cells exposed to stress. Furthermore, elevated PRINS expression in the epidermis may contribute to psoriasis susceptibility.

In the human genome, a large part of the transcriptional output is constituted of RNAs that lack protein-coding capacity (1). In recent years, much data have accumulated showing that numerous nontranslatable, noncoding RNAs are synthesized in different cells. Surprisingly, many of these noncoding RNAs that were previously thought to be functionless have been found to be developmentally regulated or expressed in a cell- or tissue-specific manner (1–3). These observations suggest that some of these transcripts are not merely a part of the large background of nonspecific transcription but have specific functions and exert their action at the RNA level. However, because there are no algorithms available for identifying non-protein-coding genes (4, 5), relatively few noncoding RNAs have been so far identified or described in detail (1). Noncoding regulatory RNAs identified to date fulfill a wide range of functions in the cells, such as regulation of chromatin modification, transcription, alternative splicing, mRNA stability, translation, or cell signaling (2, 6). Accumulating evidence suggests that the abnormal expression of various noncoding RNAs is associated with disease conditions (7–9), therefore it is of pivotal interest to study the functions of RNA genes both in physiological and in pathological conditions.

Psoriasis is a hyperproliferative, inflammatory skin disease, affecting ~2–3% of the Caucasian population (OMIM 177900, www.ncbi.nlm.nih.gov). The symptoms may appear in various forms, but either mild or severe forms of psoriasis affect the quality of life tremendously. Psoriatic patients experience disability at least at a level equivalent to that of patients with angina or hypertension (10). It is generally accepted that both genetic and environmental factors contribute to the precipitation of psoriatic lesions. It has been shown clearly that multiple genes are involved in the development of symptoms and that the disease is genetically heterogeneous (11, 12). Therefore, there may be yet unidentified factors in the human genome which contribute to psoriasis susceptibility.

Hyperproliferation of keratinocytes in the psoriatic plaques is triggered by infiltrating T-lymphocytes at the dermal-epidermal junction (13). Keratinocytes in the uninvolved skin of psoriatic patients have an inherent sensitivity to proliferative signals, and this elevated sensitivity plays a crucial role in the development of psoriatic lesions (14, 15). Hence, identification of differentially expressed genes in the uninvolved epidermis of psoriatic patients compared with healthy epidermis may reveal novel psoriasis susceptibility factors. Therefore, we performed a differential display analysis, in which the transcription profiles of psoriatic uninvolved epidermis and healthy epidermis samples were compared. Besides genes encoding proteins with characterized functions, the study identified a novel noncoding RNA gene, PRINS (Psoriasis susceptibility-related RNA gene Induced by Stress) showing elevated expression in psoriatic uninvolved epidermis, representing a putative psoriasis susceptibility-associated non-protein-coding transcript.
Materials and Methods

Human Tissue Samples—Skin biopsies (6-mm punches) were taken from uninvolved and involved areas of psoriatic patients. Control skin biopsies were obtained from healthy individuals undergoing plastic surgery. After removal of the subcutaneous tissue, skin biopsies were incubated overnight at 4 °C in Dispase solution (grade II, Roche Applied Science). On the following day, the epidermis was separated from the dermis.

Tissue samples from various human organs were taken from patients who underwent different operations at the Department of Surgery, University of Szeged. Only uninvolved, healthy tissues were used for RNA isolation.

All tissue samples were taken with the patients’ informed consent and the approval of the local ethics committee.

Differential Display—Total RNA was isolated from epidermal samples using TRIzol reagent (Invitrogen). The differential display experiment was carried out as described previously (16). 200 ng of total RNA was reverse transcribed using three different one-base-anchored H-T11-M primers (where M may be G, A, or C) of the RNAimage kit (GeneHunter Corporation, Nashville, TN) according to the manufacturer’s instructions. Two μl of the reverse transcription reactions were PCR amplified with the arbitrary H-AP1 primer of the GeneHunter kit in the presence of 0.04 MBq of [α-32P]dCTP. PCR amplification was carried out according to the GeneHunter manual. A portion of the resulting PCR products (3.5 μl) was mixed with loading dye and electrophoresed in 6% acrylamide DNA sequencing gels. Gels were dried and exposed to x-ray film for 1–3 days. Selected bands were excised from the dry sequencing gel, heated in 100 μl of water, cooled, and centrifuged briefly. The supernatant was transferred to a fresh centrifuge tube. For reamplification of differentially expressed fragments, 4 μl of the supernatant was used in PCR amplification with the H-AP1 primer and with one of the H-T11-M primers. Sizes of the amplified fragments were checked on agarose gels. Bands having the correct size were excised. After ethanol precipitation, the ends of isolated fragments were filled in using T4 DNA polymerase and the dNTP set from MBI Fermentas. PCR was carried out as described previously (16). 1 μl of PCR product was run on 1% agarose gel, stained with ethidium bromide, photographed, and evaluated using a Kodak Edas 290 densitometer and Kodak one-dimensional Digital Science software (Scientific Imaging Systems, New Haven, CT).

Table 1

| Forward primer | Reverse primer | Detection/probe |
|----------------|----------------|-----------------|
| Rab10          | CATGATTGTTGAAAAAGGACAAC | CCAATCTTCTTTCTAAGAACAAC | agarose gel electrophoresis |
| Fibronectin     | AGGACCAATTTCATATTACGCAAC | TCTCATACACTGTAGTATGAGCGGTGTAAT | agarose gel electrophoresis |
| PRINS exon 2    | GCCGCTGACTAGCTGACGCAAC | TCATCGAGCTGAGTGAATGCAGGCG | agarose gel electrophoresis |
| PRINS exon 1    | AGAGATGCGCAGTGCTTTT | ATTTCCGGTGGAGATGAGAG | agarose gel electrophoresis |
| 18S            | CCAGTACCACATCCAGAAGAA | GCTGGATATTCCCGCCGCT | agarose gel electrophoresis |

1 The abbreviations used are: FCS, fetal calf serum; HSV-1, herpes simplex virus type 1; IFN, interferon; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PRINS, psoriasis susceptibility-related RNA gene induced by stress; RT, reverse transcription; siRNA, small interfering RNA.

Cell Cultures—The spontaneously immortalized human keratinocyte cell line, HaCaT, kindly provided by Dr. N. K. Heidelburg (Germany), was cultured and synchronized as described previously (20).

For the specific inhibition of RNA polymerase II, subconfluent HaCaT cells were treated with 50 μg/ml α-amanitin (Roche Applied Science) for 5 or 24 h. Subconfluent HaCaT cells were incubated for 12 h with 1 or 3 μM concentrations of the specific RNA polymerase III inhibitor, tagetitoxin (Epigenetic Technologies, Madison, WI). HaCaT cells were then stimulated with 10 ng/ml recombinant human interferon (IFN)-γ or the combination of 1 ng/ml IFN-γ, 1 ng/ml granulocyte-macrophage-colony-stimulating factor, and 0.3 ng/ml interleukin-1 for 24 h. 5′-amino-2′-deoxyuridine (Roche Applied Science) was added to the medium to block DNA synthesis. HaCaT cells were incubated in Dispase solution (grade II) overnight at 4 °C, and on the following day the epidermis was separated from the dermis. The epithelial cells were placed in TRIzol reagent and frozen at −70 °C.

For inhibition of translation, HaCaT cells were treated with 20 μg/ml cycloheximide (Sigma), and cells were harvested after the indicated times.

Reverse Transcription-PCR (RT-PCR) —Total RNA was isolated using TRIzol reagent, according to the manufacturer’s instructions. Complementary DNA was generated from 1 μg of total RNA using the First Strand cDNA Synthesis Kit (MBI Fermentas) in a final volume of 20 μl. After reverse transcription, amplification was carried out using Taq DNA polymerase and the dNTP set from MBI Fermentas. PCR was carried out as described previously (16). 1 μl of PCR product was run on 1% agarose gel, stained with ethidium bromide, photographed, and evaluated using a Kodak Edas 290 densitometer and Kodak one-dimensional Digital Science software (Scientific Imaging Systems, New Haven, CT).

Reverse Southern Blot—pSK vectors containing the cloned differential display fragments were digested with XbaI and XhoI (MBI Fermentas) and cloned into a SmaI-digested pSK vector, using the T4 DNA ligase (MBI Fermentas).

Organotypic Cultures of Skin Specimen—Skin explants from healthy controls who underwent plastic surgery or from uninvolved skin of psoriatic patients were cultured as described previously (18, 19). Briefly, skin specimens were put on cellulose acetate/cellulose nitrate membrane/support liquid interface during the culture period. The explants were cultured at 37 °C at 5% CO2 atmosphere for 3 days. Tissue specimens were then incubated in Dispase solution (grade II) overnight at 4 °C, and on the following day the epidermis was separated from the dermis. The epithelial cells were placed in TRIzol reagent and frozen at −70 °C.

For the specific inhibition of RNA polymerase II, subconfluent HaCaT cells were treated with 50 μg/ml α-amanitin (Roche Applied Science) for 5 or 24 h. Subconfluent HaCaT cells were incubated for 12 h with 1 or 3 μM concentrations of the specific RNA polymerase III inhibitor, tagetitoxin (Epigenetic Technologies, Madison, WI). HaCaT cells were then cultured and total RNA was isolated using TRIzol reagent. For in vitro expression experiments, subconfluent cultures of HaCaT cells were used 24 h after passaging. For ultraviolet (UV)-B irradiation, cells were washed with phosphate-buffered saline and subjected to UV-B irradiation (110 mJ/cm2) in phosphate-buffered saline at room temperature. Immediately after irradiation, phosphate-buffered saline was aspirated, and culture medium was added to the culture dishes. Cells were harvested at 0, 3, 6, 12, and 24 h after UV-B exposure. For viral infection, HaCaT cells were infected with herpes simplex virus type 1 (HSV-1, strain COS) at a multiplicity of infection of 0.01 plaque-forming unit/cell, and cells were harvested after the indicated times. Mock infected cells were treated similarly without the addition of virus. For inhibition of translation, HaCaT cells were treated with 20 μg/ml cycloheximide (Sigma), and cells were harvested after the indicated times.

Reverse Transcription-PCR (RT-PCR) —Total RNA was isolated using TRIzol reagent, according to the manufacturer’s instructions. Complementary DNA was generated from 1 μg of total RNA using the First Strand cDNA Synthesis Kit (MBI Fermentas) in a final volume of 20 μl. After reverse transcription, amplification was carried out using Taq DNA polymerase and the dNTP set from MBI Fermentas. PCR was carried out as described previously (16). 1 μl of PCR product was run on 1% agarose gel, stained with ethidium bromide, photographed, and evaluated using a Kodak Edas 290 densitometer and Kodak one-dimensional Digital Science software (Scientific Imaging Systems, New Haven, CT).

RNA Interference Vector Construction and Transfection—After analyzing the secondary structure using the RNA folding server mfold (www.bioinfo.rpi.edu/applications/mfold/rna/), three different 19-mer target sequences were selected for the sequence of PRINS. Target sequences were aligned to the human genome data base in a BLAST search to ensure that the chosen sequences would work in a...
were selected in the presence of 200 μg/ml hygromycin, and several independent clones were picked up from each transformant and were analyzed further by real time RT-PCR for evaluation of the gene-specific silencing of PRINS in the cells and by MTT (Sigma) assay to study the effect of PRINS silencing on cell viability.

MHaCaT keratinocytes were seeded in wells of a 96-well plate at a density of 3 × 10^3 cells/well. Cells were grown either in serum-free medium or in the presence of 10% fetal bovine serum for 168 h. Medium was replaced with RPMI without phenol red and 50 μg of MTT was introduced into each well and incubated for 4 h at 37 °C. Living cells degrade MTT by mitochondrial succinate dehydrogenase, resulting in MTT formazan. The converted dye was solubilized with acidic isopropyl alcohol (0.04M HCl in absolute isopropyl alcohol). The optical density of the wells was determined using a microplate reader (Multiscan EX, ThermoLabystems, Vantaa, Finland) at 540 nm.

RESULTS

Differential Display and Reverse Southern Blot Reveals Differentially Expressed Genes in Psoriatic Uninvolved Epidermis—To identify gene expression differences in psoriatic uninvolved versus healthy epidermis, we performed a differential display analysis using two healthy and two uninvolved psoriatic epidermis samples. 25 bands that showed consistent difference in the pairwise comparison of the samples were cut out from the acrylamide gel. The DNA content of the gels was removed with boiling, the isolated fragments were multiplied by PCR using the primers for the differential display experiments, and after blunting, they were inserted to pSK vector. In the next round of evaluation, a reverse Southern blot technique was applied to select clones for further analysis. Fragments were cut out from the pSK vector, run in duplicate on agarose gels, blotted to nylon membranes, and hybridized with the radioactive labeled cDNAs from the healthy and from the uninvolved psoriatic epidermises. Fig. 1A illustrates that differential expression was confirmed for three samples (samples 1, 2, and 3), whereas sample 4 showed about the same signal intensity with the two probes. The expression of RAB10, fibronectin, and AK022045 transcripts (corresponding to samples 1, 2, and 3, respectively) was analyzed by RT-PCR by using the primer pairs listed in Table I.

The Identified Transcripts Show Differential Regulation during Proliferation/Differentiation of Keratinocytes—Because psoriasis is a skin disease characterized by abnormal proliferation and differentiation of epidermal keratinocytes, we aimed to investigate whether the expression of the identified transcripts was affected by the proliferation/differentiation state of the keratinocytes. For this purpose, we used synchronized HaCaT keratinocyte cultures, which represent a model for keratinocyte proliferation and differentiation (20). In this model system, serum-starved, contact-inhibited cells (0 h) resemble suprabasal nonproliferating differentiated keratinocytes of normal epidermis, whereas the highly proliferative HaCaT cells after release from cell quiescence (24–168 h) resemble the activated, differentiated, transiently amplifying keratinocytes.

RT-PCR results revealed (Fig. 1B) that RAB10 mRNA was expressed approximately at the same level in different stages of keratinocyte proliferation/differentiation. In contrast to RAB10, fibronectin showed a highly regulated pattern of expression: the serum-starved, contact-inhibited HaCaT cells expressed a very low level of fibronectin mRNA, which increased dramatically with time after passaging and serum readdition. The highest levels of total fibronectin mRNA coincided with the highest proliferation rate of HaCaT cells (48 and 72 h). Because the primer pair we used to detect the fibronectin mRNA abundance was designed to border the EDA motif of fibronectin, we were able to detect both the EDA- and EDA- isoforms of fibronectin (Fig. 1B) as well as changes in the ratio of these two isoforms (22). The novel cDNA, AK022045, showed the highest level of expression in the serum-starved, contact-inhibited HaCaT keratinocytes (0 h sample), and as the cells started to proliferate because of passaging and serum readdition, the abundance of AK022045 cDNA dropped dramatically (24–96 h samples) and did not seem to increase even after 168 h, in the confluent culture (Fig. 1B).

Results of the in Silico Analysis of the Novel Transcript Are Indicative for a Noncoding RNA Gene—To analyze further the structural characteristics of the novel cDNA, we performed
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| Position* and sequence of forward primers | Position and sequence of reverse primers | Expected length of amplicon |
|------------------------------------------|------------------------------------------|-----------------------------|
| −554 TTTAGTAAACATCTACCGAGCAGT            | +302 AAAACAAATGGTGCGCTGAG               | 846                         |
| −1141 TGCTGGCAGATAATGCTTTGG              | −370 CCAGCACAACCCGACATAAGT              | 771                         |
| −1483 CTTTTCCCTTTCCGAGACAA              | −1118 CAGGCCAAAACAGATTACCTGC            | 365                         |
| −2389 ACAGGGTGGTGCCTGGTGAC              | −1464 TTGTCCTGGAAAAGGAAAGGA            | 905                         |

*Relative to the first nucleotide in the sequence of the cDNA with accession number AK0222045.

Fig. 2. The structure of the full-length PRINS transcript in HaCaT keratinocytes. A, total RNA was isolated from HaCaT keratinocytes, reverse transcribed, and RT-PCRs were carried out using the primer pairs listed in Table II. Primers were designed for the already known sequence of the transcript with GenBank accession number AK0222045 and for the genomic sequence proximal to the 5’-end of it (M, DNA molecular weight marker). The most proximal primer pair did not result in an amplicon with the expected size, suggesting that the full-length transcript in HaCaT cells is at least 1,483 bp but no more than 2,369 bp longer than the originally sequenced cDNA, AK0222045. B, comparison of the cDNA and genomic sequences revealed that the AK0222045 transcript consists of two exons. Homology searches revealed the presence of two Alu elements within the PRINS sequence and a sequence that showed ~70% similarity with an element for developing thermotolerance in Tetrahymena thermophila (Fig. 2B). These characteristics and the fact that the transcript could not be translated in silico into any protein sequence prompted us to hypothesize that AK0222045 is a noncoding gene. Because we detected a robust overexpression of this gene in psoriatic uninvolved epidermis and a stress-induced expression in HaCaT keratinocytes, the full-length transcript was named Psoriasis Susceptibility-Related RNA Gene Induced by Stress (PRINS).

Quantitative RT-PCR Experiments on Independent Psoriatic Involved and Uninvolved Epidermis Samples and in Organotypic Cultures Indicate That PRINS Is a Factor Related to Psoriasis Susceptibility—Results of the differential display and the reverse Southern experiments have clearly indicated that PRINS is overexpressed in the uninvolved psoriatic epidermis relative to healthy epidermis. To confirm these results on several independent samples and to get insights into the possible role of PRINS in psoriasis, we performed real time RT-PCR analysis on independent healthy (n = 14), psoriatic uninvolved (n = 13) and involved (n = 14) epidermal samples. Results of the real time RT-PCR analysis confirmed that PRINS was overexpressed in the psoriatic uninvolved epidermis (24-fold compared with normal epidermis, p < 0.01; Fig. 3A). The abundance of PRINS transcript in the psoriatic plaques did not reach the level observed in uninvolved psoriatic epidermis; however, it was still significantly, 12-fold higher compared with healthy epidermis (p < 0.05; Fig. 3A). Fig. 3B shows a comparison of PRINS expression in the paired involved and uninvolved epidermis samples from eight psoriatic patients. In six of eight patients, higher levels of PRINS transcripts were detected in the uninvolved epidermis compared with psoriatic lesions (Fig. 3B). However, PRINS was up-regulated in both involved and uninvolved epidermis in all studied patients compared with healthy controls. Interestingly, in two patients (P#4 and P#5), similar levels of PRINS expression could be observed in involved and uninvolved epidermis (Fig. 3B).

To investigate further the connection between PRINS expression and psoriasis, we studied the expression of PRINS in organotypic cultures by real time RT-PCR. Healthy (n = 5) and psoriatic uninvolved (n = 5) epidermis specimens were treated with T-cell lymphokines, either with IFN-γ or a mixture of IFN-γ, interleukin-3, and granulocyte-macrophage colony-stimulating factor, the latter combination known to induce keratinocyte hyperproliferation in uninvolved psoriatic but not in normal keratinocytes in vitro (14). T-lymphokine treatment did not substantially affect the expression of PRINS in healthy epidermis (data not shown). In contrast, in uninvolved psoriatic epidermis, treatment with the T-lymphokine mixture resulted in a 5-fold decrease in PRINS expression compared with the untreated uninvolved epidermis (Fig. 3C). IFN-γ treatment...
alone did not change PRINS expression in the uninvolved epidermis (Fig. 3C). The high expression of PRINS in the uninvolved, but lower expression in the lesional epidermis of psoriatic patients, and the decrease of PRINS RNA level in uninvolved psoriatic epidermis, but not in normal epidermis in response to T-lymphokines suggests that PRINS overexpression plays a role in psoriasis susceptibility and not in the precipitation of psoriatic symptoms.

**PRINS Is Transcribed by RNA Polymerase II**—PRINS was identified from a differential display experiment that was performed using arbitrary oligo(dT) primers, suggesting that the transcript may harbor a poly(A) tail indicative of transcription by RNA polymerase II. However, the presence of internal RNA polymerase III promoters in the sequence would enable transcription by RNA polymerase III. To determine which RNA polymerase transcribes PRINS, we performed in vitro experiments on HaCaT keratinocytes using a specific RNA polymerase II inhibitor, α-amanitin, and a specific RNA polymerase III inhibitor, tagetitoxin (23).

Subconfluent HaCaT cells were treated with 50 μg/ml α-amanitin, which has an effect only on RNA polymerase II but not on RNA polymerase III at this concentration (24). Results of real time RT-PCR analysis revealed that the expression of PRINS dropped dramatically after a 5-hour coincubation with α-amanitin (10-fold compared with the untreated control), and incubation for 25 h further reduced the expression of PRINS (33-fold compared with the untreated control) (Fig. 4A).

In contrast to α-amanitin, tagetitoxin (1 and 3 μM for 6 and 12 h) did not change PRINS expression significantly compared with the untreated controls (Fig. 4B). To confirm the effect of tagetitoxin at the applied concentrations, the expression of a well-characterized RNA polymerase III transcribed gene, 7SL, has also been studied. Real time RT-PCR analysis showed that tagetitoxin decreased the expression of the 7SL gene at both concentrations, indicating that RNA polymerase III was effectively inhibited by these concentrations of tagetitoxin (data not shown).

**PRINS Is Expressed in Various Human Tissues, and the Level of Expression Shows Great Variability**—To investigate the tissue specificity of PRINS, the absolute amount of PRINS...
RNA was determined by quantitative real-time RT-PCR in healthy tissue samples from various human organs. PRINS was found to be expressed in all of the studied organ types (Fig. 5). Interestingly, the level of expression differed to a great extent in different organs. The lowest level of PRINS RNA was detected in cardiac muscle, whereas the highest level was seen in veins. The level of expression was 18-fold higher in veins than in cardiac muscle. It is interesting to note that compared with all tissues studied, the abundance of the transcript was relatively low in the healthy epidermis.

**Serum Deprivation with or without Contact Inhibition Induces PRINS Expression in HaCaT Keratinocytes**—In our initial experiments, using the model system of synchronized HaCaT keratinocytes, the AK022045 cDNA was expressed abundantly in nonproliferating, differentiated cells, whereas its expression decreased in proliferating cells (Fig. 1). To confirm further the changes in PRINS expression in different proliferation/differentiation states of keratinocytes, we performed a quantitative real-time PCR analysis of PRINS expression in this model system. Our results confirmed that PRINS is expressed at a high level in serum-starved, contact-inhibited cells, whereas in proliferating cells, the abundance of PRINS RNA drops dramatically. After 12 h, a 5-fold and after 24 h, a 50-fold decrease could be observed compared with the serum-starved, contact-inhibited cells (Fig. 6A). During the 36–168 h period of culture, the abundance of the PRINS transcript increased slightly, but it was still 8–16-fold lower than the expression observed in the serum-starved, contact-inhibited (0 h) cells. These findings correlate well with the high expression of PRINS in uninvolved psoriatic epidermis but relatively lower expression in the psoriatic lesions with keratinocyte hyperproliferation.

In addition, these observations also suggested that the high expression of PRINS was connected to the stressed state of HaCaT keratinocytes induced by contact inhibition and serum starvation. Therefore, we investigated whether serum starvation alone, as a stress signal, also induces PRINS. To this end, HaCaT keratinocytes were cultured in the presence of 10% FCS or in serum-free medium for 24 or 72 h, and the expression of PRINS was analyzed by quantitative real-time PCR. In serum-starved cells, the abundance of PRINS RNA increased up to 3-fold after 24 h and 6-fold after 72 h compared with the 0 h controls (Fig. 6B), whereas in the presence of serum, PRINS expression increased only in the 72 h confluent culture. These results indicate that nutrient deprivation, representing a stress situation for keratinocytes, induces the expression of PRINS.

**Environmental Stress Factors and Translation Inhibition Induce the Expression of PRINS**—The inducibility of PRINS upon serum starvation and contact inhibition prompted us to hypothesize that PRINS is a stress-inducible RNA gene. Therefore, we aimed to investigate whether PRINS is induced by other types of stress cues relevant in skin.

UV radiation is one of the most important environmental stress factors, and its main target is the epidermis. UV-B (290–320 nm) is considered to be the causative agent of many of the harmful effects attributed to UV, causing DNA damage by cross-linking and modifying the pattern of gene expression (25). The effect of UV-B exposure (110 mJ/cm²) on PRINS expression was examined 3, 6, 12, and 24 h after the irradiation.
of HaCaT cells. Results of quantitative real time PCR analysis showed that UV-B irradiation significantly induced PRINS expression already after 3 h \((p < 0.05; \text{Fig. 7A})\), and peak expression was observed 24 h after irradiation.

To investigate the effect of viral infection on PRINS expression, HaCaT cells were infected with HSV-1 at a multiplicity of infection of 0.01 plaque-forming unit/cell, and samples were collected at the indicated times after virus adsorption. C, the translational inhibitor cycloheximide (20 \(\mu\)g/ml) was added to HaCaT cells, and samples were collected after the indicated times. PRINS expression was determined by quantitative real time RT-PCR. Data are indicated as -fold expression compared with the time-matched untreated controls. Values represent means of \(n=4\) independent experiments \(\pm\) S.E. \((^*p < 0.05, ^{* *}p < 0.01, \text{Student's} t\ test)\).

Viral infection causes complex changes in host cell biosynthesis, including inhibition of translation of selective host mRNAs (26). To investigate whether direct translational inhibition affects the expression of PRINS, HaCaT keratinocytes were treated with cycloheximide, an inhibitor of protein synthesis, at 20 \(\mu\)g/ml final concentration, and the abundance of PRINS RNA was determined by quantitative real time PCR. PRINS expression was induced rapidly by cycloheximide treatment, already after 30 min \((p < 0.01; \text{Fig. 7C})\), and it was still elevated after 12 h \((\text{Fig. 7C})\).

**Gene-specific Silencing of PRINS Decreases the Viability of Cells Exposed to Stress—**Having established the inducibility of PRINS in response to a wide range of skin-relevant stress factors, we addressed the question of whether PRINS has any functional role in cellular stress responses. To this end, we knocked down the expression of PRINS in HaCaT keratinocytes using a vector-based RNA interference method, and we examined the stress tolerance of the transfected HaCaT cells. To evaluate the efficacy of the siRNA-mediated PRINS depletion, the PRINS RNA level was determined by quantitative real time PCR analysis in transfected HaCaT cells. As negative control, we used a vector containing the scrambled sequence of one of the studied siRNAs of PRINS. In addition to the basal level of PRINS RNA, we also determined the level of PRINS RNA after 72 h of serum starvation in the transfected cells. One of the siRNAs tested (AK696) significantly down-regulated PRINS expression in HaCaT cells \((\text{Fig. 8A})\), indicating the effectiveness of this siRNA on degrading PRINS RNA. Moreover, the AK696 siRNA also repressed the induction of PRINS in serum-starved cells \((\text{Fig. 8A})\). After 72 h of serum starvation, the abundance of PRINS RNA was still 1.8-fold lower in the AK696 siRNA-transfected cells than in the controls.

To investigate whether PRINS affects the viability of cells in stress conditions, the siRNA-transfected HaCaT keratinocytes were cultured in the presence of 10% FCS or in serum-free medium for 168 h, and cell viability was measured by MTT assay. In the presence of serum, cell viability was not affected
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significantly by the down-regulation of PRINS (Fig. 8B). However, after 168 h, cell viability was decreased significantly in the serum-starved AK696 siRNA-transfected cells as compared with the controls containing the scrambled siRNA (p < 0.001) as well as compared with the AK696 siRNA containing cells cultured in the presence of serum (p < 0.001). These findings demonstrate that the down-regulation of PRINS impairs cell viability in serum-starved cells, suggesting that PRINS is essential for the survival of keratinocytes under stress conditions.

DISCUSSION

Using differential display analysis to compare gene expression in uninvolved epidermis of psoriatic patients and epidermis of healthy individuals, we identified several differentially expressed transcripts that may play a role in psoriasis susceptibility. Some of the identified transcripts encode proteins with already characterized functions, such as the extracellular matrix protein, fibronectin. The high expression of fibronectin in psoriatic uninvolved epidermis is in good agreement with previous findings demonstrating the expression of fibronectin at the dermal-epidermal junction of psoriatic uninvolved but not of normal epidermis (15, 21). Another transcript showing elevated expression in psoriatic uninvolved epidermis was RAB10, a member of the ras oncogene family. Elevated RAB10 gene expression has also been found previously in other pathological conditions such as hepatocellular carcinoma (27) and melanoma (28).

Beside genes with already known and characterized functions, we also identified a yet uncharacterized transcript showing elevated level in psoriatic uninvolved epidermis samples. Structural analysis of the novel transcript revealed a striking feature: the high density of stop codons in all three reading frames and a resulting lack of an extensive open reading frame. In silico translation of the sequence did not result in any protein product, suggesting that the novel transcript lacks protein-coding capacity and functions as a noncoding RNA. Homology studies on the full-length cDNA sequence also supported this hypothesis because the sequence contains an element with high level homology to a heat shock element in a small noncoding RNA, G8. Moreover, it also harbors Alu repetitive elements, similarly to already characterized noncoding RNA genes such as the neurospecifically expressed noncoding human RNA gene, BC200 (24). Based on its high expression in psoriatic uninvolved epidermis and the observed stress inducibility in in vitro experiments, we named the novel transcript PRINS (Psoriasis-susceptibility-Related RNA Gene Induced by Stress). PRINS consists of two exons, and it is located on chromosome 10. Using specific transcription inhibitors we showed that PRINS is transcribed by RNA polymerase II, despite the presence of internal RNA polymerase III promoters in its sequence. The full-length transcript expressed in HaCaT cells is longer than the cDNA detected in human embryonic tissue (GenBank accession number AK022045), and a putative TFIIB transcription factor binding site has been identified on the genomic sequence proximal from the possible transcription start site. Based on these characteristics of structure and transcription, PRINS belongs to the group of recently described mRNA-like noncoding RNA genes (including e.g. BORG, H19, PCGEM1), which are spliced, polyadenylated mRNA molecules containing a high density of stop codons and lacking an extensive open reading frame (7, 29, 30).

To investigate the tissue specificity of PRINS, we studied its expression pattern in various human tissues. PRINS RNA was detectable in all human tissue samples we studied, and the level of its expression showed a great variability. These findings suggest that PRINS is a ubiquitously expressed transcript in the human body; however, its expression level is regulated in a tissue-specific manner. It is for future studies to identify elements responsible for the fine tuning of tissue-specific expression of PRINS. Notably, the highest level of PRINS transcripts could be detected in veins. Psoriatic lesional skin is characterized by an expansion of the superficial dermal microvasculature (31, 32), and it has been shown that microvascular changes occur early in the development of psoriatic lesions (31). The high expression of PRINS in veins indicates that endothelial cells may express PRINS, which might regulate elements of angiogenesis-associated pathways. To support this hypothesis further studies comparing the expression of PRINS in the dermis of healthy, uninvolved and involved skin need to be performed.

The elevated expression of PRINS in psoriatic uninvolved epidermis was further confirmed by real time PCR analysis performed on several independent samples. Interestingly, the expression of PRINS in psoriatic lesions was higher compared with healthy epidermis but lower than in the uninvolved epidermis. Moreover, lymphokine treatment, known to induce hyperproliferation of uninvolved psoriatic keratinocytes, decreased the PRINS RNA level in psoriatic uninvolved but not in healthy epidermis. These results suggest that PRINS overexpression plays a role in psoriasis susceptibility and not in the precipitation of psoriatic symptoms. Moreover, these observations are in agreement with previous results showing that keratinocytes in the uninvolved skin of psoriatic patients differ from healthy keratinocytes in their responses to external stimuli (14, 21, 22). The overexpression of PRINS in the psoriatic uninvolved epidermis may reflect an altered regulatory extracellular milieu, but it is also possible that PRINS plays a regulatory role in the hyperproliferation of keratinocytes in psoriasis. Thus, PRINS may contribute to psoriasis susceptibility as a modifier gene. Interestingly, in two patients, PRINS transcript levels in the uninvolved epidermis were similar to those observed in psoriatic lesions, thus PRINS expression might be a newly identified factor reflecting the clinical heterogeneity of psoriasis. This observation as well as the relatively large interindividual differences suggest that PRINS expression may be influenced by a yet unknown disease-associated factor in the epidermis of psoriatic patients.

In synchronized HaCaT cells, a model system for keratinocyte proliferation and differentiation, PRINS expression was high in the serum-starved, contact-inhibited, nonproliferating cells, but much lower in proliferating cells, in good correlation with its high expression in vivo in psoriatic uninvolved epidermis and lower expression in the psoriatic lesions characterized by the hyperproliferation of keratinocytes.

The up-regulation of PRINS in serum-starved, contact-inhibited cells suggests that the high level of PRINS RNA is associated with the stressed state of cells. We showed that serum starvation alone is sufficient to induce PRINS expression, even without contact inhibition. These findings as well as sequence homologies to stress-induced transcripts such as the heat shock-inducible RNA, G8, and Alu elements, prompted us to hypothesize that PRINS is induced also by other types of stress stimuli. Indeed, skin-relevant environmental stress factors, such as UV-B irradiation and viral infection (HSV-1), increased the RNA level of PRINS in HaCaT keratinocytes. In addition, direct translational inhibition by cycloheximide also induced PRINS expression. It is remarkable that the stress inducibility of PRINS resembles the regulation of short transcripts derived from repetitive elements including Alu, which are induced by various stress signals (33–35) and translation inhibition (33, 36, 37). The similarity in the regulation of short Alu transcripts and the Alu-element harboring PRINS in response to stress
stimuli suggests that induction of PRINS is a part of the general cellular stress response. To explore whether PRINS plays a functional role in the stress response of keratinocytes, we used RNA interference, a powerful tool to knock down specific gene expression. Down-regulation of PRINS by a specific siRNA significantly decreased the viability of cells after serum starvation, but not under normal serum conditions. This observation suggests that PRINS exerts its function in keratinocytes exposed to stress and might have a protective function against stress-induced cell death.

Despite the growing number of identified noncoding RNA genes, their biological function is still largely unknown. In addition to Alu elements, several noncoding RNAs, including polyadenylated and spliced transcripts, have been shown to be induced by different stress signals. G8, a small RNA gene showing homology to PRINS, is induced by heat shock in *T. thermophila* (38), the gadd7 RNA is induced by DNA-damaging agents (39), whereas adapt15 and adapt33 RNAs are induced in response to oxidative stress (40, 41). Several noncoding transcripts have been shown to be expressed abnormally in various human diseases, which emphasizes the importance of understanding their functions in normal cells. MALAT-1 is overexpressed in non-small cell lung cancer (8), and PCGEM1 and DD3 show significant overexpression in prostate cancer (7, 42). Most of the noncoding RNAs are supposed to regulate the expression of other genes under certain conditions (2, 3). We hypothesize that PRINS may also function as a "riboregulator," modifying the expression of other genes involved in the proliferation and survival of cells exposed to stress. Further studies of this gene will provide new insights into the complex stress response pathways as well as into the role of noncoding RNAs in the pathogenesis of human diseases.

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