B4137

IDENTIFICATION AND CHARACTERIZATION OF A NOVEL PSORIASIS-SUSCEPTIBILITY-RELATED, NON-CODING RNA GENE, PRINS

Ph.D. dissertation

Enikő Sonkoly M.D.

Supervisor:

Márta Széll Ph.D.

Department of Dermatology and Allergology

University of Szeged, Szeged, Hungary

2005

TABLE OF CONTENTS

LIST OF PUBLICATIONS

I. **E Sonkoly**, Zs Bata-Csorgo, A Pivarcsi, H Polyanka, A Kenderessy-Szabo, G Molnar, K Szentpali, L Bari, K Megyeri, Y Mandi, A Dobozy, L Kemeny, and M Szell: Identification and characterization of a novel, psoriasis-susceptibility-related non-coding RNA gene, PRINS. ***Journal of Biological Chemistry,*** *in press (published April 26, 2005 as doi:10.1074/jbc.M501704200).*

II. A Pivarcsi, M Gombert, MC Dieu-Nosjean, A Lauerma, R Kubitza, S Meller, J Rieker, A Muller, LD Cunha, A Haahtela, **E Sonkoly**, WH Fridman, H Alenius, L Kemeny, T Ruzicka, A Zlotnik B Homey: CCL18, an atopic dermatitis associated and dendritic cell-derived chemokine, is regulated by staphylococcal products and allergen exposure. ***Journal of Immunology*** *2004;173(9):5810-7.*

III. T Vág, **E Sonkoly**, S Kárpáti, B Kemény, J Ongrádi: Avidity of antibodies to human herpesvirus 7 suggests primary infection in young adults with pityriasis rosea. ***Journal of the European Academy of Dermatology and Venereology,*** *2004; 18(6):738-40.*

IV. T Vág, **E Sonkoly**, B Kemény, S Kárpáti, A Horváth, J Ongrádi: Familiar occurrence of papular-purpuric „gloves-and-socks" syndrome with human herpesvirus-7 and human parvovirus B19 infection. ***Journal of the European Academy of Dermatology and Venereology,*** *2004;18(5):639-41.*

V. M Széll, **E Sonkoly,** Zs Bata-Csörgő, A Pivarcsi, H Polyánka, A Kenderessy-Szabó, K Szentpáli, G Molnár, L Kemény: PRINS: egy új nem kódoló RNS gén azonosítása, expressziójának vizsgálata pikkelysömörben, valamint különböző humán szervekben és szövetekben. ***Bőrgyógyászati és Venerológiai Szemle,*** *2004; 80: 255-260.*

VI. T Vág, **E Sonkoly**, B Kemény, S Kárpáti, A Horváth, J Ongrádi: Az emberi 7-es herpesvírus újabb szerepének vizsgálata bőrbetegségekben. ***Orvosi Hetilap,*** *2003; 144: 1623-1629.*

1. INTRODUCTION

1.1. Psoriasis

1.1.1 Clinical and histological features of psoriasis

Psoriasis is a hyperproliferative, inflammatory skin disease of multifactorial origin, affecting approximately 2-3% the population worldwide. The symptoms may appear in various forms but either mild or severe forms of psoriasis affect the quality of life tremendously. Psoriasis is a lifelong inflammatory disease with spontaneous remissions and exacerbations (1), and patients experience disability at least at a level equivalent to that of patients with angina or hypertension (2).

Clinically, the cardinal features of psoriatic lesions are erythema, induration and scaling. Involved areas are usually well demarcated and distributed in a characteristically symmetrical manner (3, 4). The disease has certain distinct but overlapping clinical phenotypes including chronic plaque lesions (psoriasis vulgaris), acute and usually self-limiting guttate type eruptions, seborrhoeic psoriasis, pustular lesions, and at least 10% of these patients develop arthritis (5). Many triggering factors initiate or exacerbate psoriasis, including bacterial pharyngitis (group A streptococci) (6), psychological stress, HIV-1 and various medications (1).

Histopathologically, plaque-stage lesions reveal significantly thickened epidermis with confluent parakeratotic scale, loss of the granular cell layer, and elongated rete ridges (Fig. 1A). The papillary dermal blood vessels appear tortuous and dilated. The inflammatory cell infiltrate consists mainly of CD4+ and CD8+ T cells in the dermis and CD8+ T cells in the epidermis, accompanied by increased numbers of dermal dendritic cells, macrophages and mast cells (1, 7).

1.1.2. Genetic studies in psoriasis

It is generally accepted that psoriasis has a strong genetic background. The disease is frequently inherited and passed from one generation to the next, but it does not follow a

stripping), psoriatic uninvolved skin shows significantly higher epidermal cell proliferation than normal skin (16). Moreover, in psoriatic uninvolved epidermis, the clonogenic basal stem-cell population has been shown to be more sensitive to T cell-derived lymphokines than basal cells derived from normal epidermis (17). T cell-derived cytokines such as interferon (IFN)-γ, interleukin (IL)-3, and granulocyte-macrophage colony stimulating factor (GM-CSF) have a more pronounced growth-stimulatory effect on keratinocytes from uninvolved psoriatic skin as compared to healthy keratinocytes (17) (Fig. 1B). These findings indicate that a key aspect of the development of symptoms is the inherent sensitivity of uninvolved psoriatic keratinocytes to exogenous proliferative signals such as mechanical stress or cytokines. This inherent sensitivity is partially due to the increased expression of α5 integrin (18) and its aberrantly produced ligands, fibronectin and the EDA^+ oncofetal fibronectin (19, 20). Recent global gene expression profiling studies have also confirmed that uninvolved skin of psoriatic patients (also known as prepsoriatic and symptomless skin) shows numerous molecular differences as compared to healthy skin (3, 21).

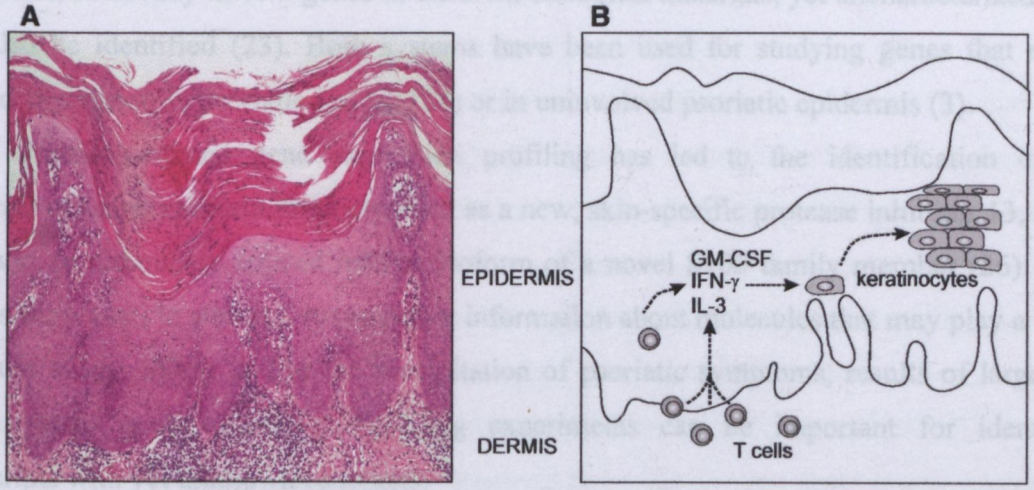

Figure 1. Histological appearance of chronic psoriatic plaques and the mechanisms leading to keratinocyte hyperproliferation in psoriatic lesions. A chronic psoriatic plaque (*A*) reveals markedly thickened skin, parakeratosis, loss of the granular cell layer and dermal infiltration of lymphocytes, dendritic cells and macrophages. Skin-infiltrating T cells in the dermis and epidermis play an important role in inducing keratinocyte hyperproliferation (*B*): lymphokines such as granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin (IL)-3 and

interferon (IFN)-γ produced by T cells stimulate the proliferation of stem cells in the basal layer of the psoriatic epidermis.

1.1.4. Detection of gene expression differences between healthy and psoriatic uninvolved epidermis: a possible way to reveal psoriasis susceptibility factors

Since keratinocytes in the uninvolved skin of psoriatic patients have an inherent sensitivity to proliferative signals derived from T cells, and this elevated sensitivity plays a crucial role in the development of psoriatic lesions (17, 18), identification of differentially expressed genes in the uninvolved psoriatic epidermis in comparison to healthy epidermis may reveal novel psoriasis susceptibility factors.

For identifying gene expression differences, there are two main methods: in "closed systems," the expression of already known and annotated genes can be compared (such as the DNA microarray technique (22)), while in "open systems," in addition to comparing the expression of already known genes in different biological materials, yet uncharacterized genes can also be identified (23). Both systems have been used for studying genes that exhibit altered expression in psoriatic lesions (24) or in uninvolved psoriatic epidermis (3).

Psoriasis-related gene expression profiling has led to the identification of new isoforms of known protein products, such as a new, skin-specific protease inhibitor 13, hurpin (25) and an alternately spliced mRNA isoform of a novel S100 family member (26). These results show that, in addition to providing information about molecules that may play a role in psoriasis susceptibility and/or the precipitation of psoriatic symptoms, results of large-scale open system gene expression profiling experiments can be important for identifying transcripts with yet unknown functions.

1.2 Non-coding RNA genes

In the human genome, a large part of the transcriptional output is constituted of RNAs that lack protein-coding capacity (27, 28). In recent years, much data has accumulated showing that various, non-translatable, non-coding RNAs are synthesized in different cells. Surprisingly, many of these non-coding RNAs that were previously thought to be functionless have been found to be developmentally regulated or expressed in a cell- or tissue-specific

SZTE Egyetemi Könyvtár SZEGED 14.

manner (27, 29, 30). These observations suggest that some of these transcripts are not solely a part of the large background of nonspecific transcription, but have a specific function and exert their action at the RNA level. In addition to housekeeping RNAs such as rRNAs, tRNAs, snRNAs and snoRNAs, a number of other non-coding RNAs have been discovered in recent years, including microRNAs and mRNA-like, larger transcripts (29). The regulatory non-coding RNAs identified to date fulfil a wide range of functions in the cells such as regulation of chromatin modification, transcription, alternative splicing, mRNA stability, translation or cell signaling (27, 31).

A large fraction of the transcriptional activity in the human genome is derived from repeat sequences that are transcribed, such as long terminal repeats, short interspersed elements (SINEs), and long interspersed elements (LINEs) (29). *Alu* repetitive elements constitute 10% of the human DNA, they belong to the group of SINEs, and are generally considered to be functionless mobile elements playing role solely in the evolution of the human genome (32). However, recent studies have shown that some of the *Alu* elements show a regulated expression pattern and thus may have functions in stress response, chromatin organization or signaling events in the early embryo (32, 33).

Relatively few non-coding RNAs have been identified or described in detail, since interest in the transcriptional activity of the genome has been focused almost exclusively on protein-coding genes. Most of the sequence related gene-finding algorithms, which have been developed to identify and define classical genes within the genomic sequence, are designed to identify open reading frames, polyadenylation signals, conserved promoter regions and splice sites typically associated with protein-coding genes (29, 34, 35). Although relevant computational and experimental approaches have now been initiated (29, 35), it is still difficult to estimate how many of these genes are present in the genome. Hence, open system, large-scale gene expression studies are still a valuable tool for identifying and characterizing novel genes that do not code for proteins but function as RNAs.

2. AIMS

The aims of our study were:

- To identify gene expression differences between the uninvolved epidermis of psoriatic patients and the epidermis of healthy individuals, in order to reveal novel psoriasis susceptibility factors
- To confirm the differential expression of the identified transcripts in psoriatic uninvolved epidermis, using several independent samples
- To investigate the regulation of the identified transcripts during keratinocyte proliferation and differentiation
- To characterize functionally the differentially expressed transcripts and to investigate their possible role in the pathogenesis of psoriasis.

3. MATERIALS AND METHODS

3.1 Human tissue samples

Skin biopsies (6 mm punches) were taken from uninvolved and involved areas of psoriatic patients with the patients' informed consent and the approval of the local ethics committee. Control skin biopsies were obtained from healthy individuals undergoing plastic surgery. After removal of the subcutaneous tissue, the skin biopsies were incubated overnight at 4°C in Dispase solution (Grade II, Roche Molecular Biochemicals, Mannheim, Germany). 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 non-involved, healthy tissues were used for RNA isolation.

Tissue samples were stored in Trizol reagent at -70°C and RNA was isolated according to the instructions of the manual (Gibco BRL, Eggstein, Germany).

3.2 Differential display

Total RNA was isolated from epidermal samples using Trizol reagent according to the instructions of the manufacturer (Gibco BRL). The differential display experiment was carried out as previously described (36). 200 ng total RNA was reverse transcribed using three different one-base-anchored H-T_{11}M primers (where M may be G, A, or C) of the RNAimage kit (GeneHunter Corporation, Nashville, TN, USA) according to instructions. Two micrograms of the reverse transcription reactions were PCR amplified with the arbitrary H-AP1 primer of the GeneHunter kit in the presence of 0.04MBq $\alpha[^{32}P]$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 re-amplification 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-T_{11}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 (MBI Fermentas, Vilnius, Lithuania) and cloned into a *SmaI* digested pSK vector, using the T4 DNA ligase (MBI Fermentas).

3.3 Reverse Southern blot

pSK vectors containing the cloned DD fragments were digested with *XbaI* and *XhoI* (MBI Fermentas), run on 1.5% agarose gels then the bands were transferred to NytranN nylon membrane (Schleicher & Schuell, Dassel, Germany). The same cDNAs (originating from healthy and psoriatic uninvolved epidermises) that were used for the DD experiments were again labeled with $\alpha[^{32}P]$dCTP, using the corresponding primers of the RNAimage kit and used as probes. Pre-hybridization and hybridization were carried out by standard methods (37) and the filters were exposed to X-ray films.

3.4 Organotypic cultures of skin specimen

The top layer of skin was prepared either from healthy control patients who underwent plastic surgery or from the uninvolved skin of psoriatic patients. The skin specimens were put on cellulose acetate/cellulose nitrate filters with 2.2 μm porosity (Millipore, Billerica, MA, USA) and placed on a stainless steel grid platform in a 25 mm^2 Petri dish. The Petri dishes were filled with DMEM (Gibco BRL) supplemented with 10% FBS, 2 mM glutamine (Gibco BRL), 100 U/ml penicillin and 100 μg/ml streptomycin (Gibco BRL). As inducing factors, either 1 ng/ml IFN-γ or the combination of 1 ng/ml IFN- γ, 1 ng/ml GM-CSF and 0.3 ng/ml IL-3 were added to the medium. The above procedure enabled the tissues to be maintained at the air/liquid interface during the culture period. The explants were cultured at 37°C at 5% CO_2 atmosphere for 3 days. Then tissue specimens were incubated in Dispase solution (Grade II, Roche Molecular Biochemicals) overnight at 4°C and on the following day the epidermis was separated from the dermis. The epidermis samples were placed in Trizol reagent and frozen at -70°C.

3.5 Cell cultures

3.5.1 Culturing of HaCaT keratinocytes

Human HaCaT cells, kindly provided by Dr. N. E. Fusenig (Heidelberg, Germany), were grown in 75cm^2 cell culture flasks (Costar, Cambridge, MA, USA) and maintained in high glucose Dulbecco's modified Eagle's medium (high glucose DMEM, Gibco, Eggstein, Germany) supplemented with 10% fetal bovine serum (FBS) (Gibco), L-glutamine, penicillin/streptomycin and fungizone (Sigma, Budapest, Hungary) at 37°C in a humidified atmosphere containing 5% CO_2.

3.5.2 Synchronization of HaCaT keratinocytes

HaCaT keratinocytes were synchronized by cultivating them at high density in the absence of serum. Cells were grown to 100% confluence in medium containing 10% FBS for 5 days, then the medium was replaced by serum-free high glucose DMEM. The cells were grown in serum free media for 1 week. The synchronized cells were trypsinized and were subsequently seeded into 75cm^2 culture flasks at a density of 5x10^3 cells/cm^2 in high glucose DMEM containing 10% FBS. Samples were taken from parallel cultures at different times after passing the cells to the high glucose DMEM containing 10% FBS.

3.5.3 RNA polymerase inhibition

For the specific inhibition of RNA polymerase II, subconfluent HaCaT cells were treated with 50 μg/ml α-amanitin (Boehringer-Mannheim, Mannheim, Germany) for 5 or 25 hours. Subconfluent HaCaT cells were incubated for 12 hours with 1 μM or 3 μM concentrations of the specific RNA polymerase III-inhibitor, tagetitoxin (Epicentre Technologies, Madison, WI, USA). HaCaT cells were harvested and total RNA was isolated using Trizol reagent (Gibco BRL).

3.5.4 Stress treatments

For *in vitro* expression experiments, subconfluent cultures of HaCaT cells were used, 24h after passaging. For UV-B irradiation, cells were were washed with PBS and subjected to

UV-B irradiation (110 mJ/cm^2) in PBS at room temperature. Immediately after irradiation, PBS was aspirated and culture medium was added to the culture dishes. Cells were harvested at 0, 3, 6, 12 and 24 hours after UV-B exposure.

For treatment with microbial and fungal compounds, HaCaT cells were co-incubated with the following compounds for the indicated times: LPS (2.5 μg/ml, purified from *Escherichia coli*; 026:B6), *Staphylococcus aureus* peptidoglycane (PGN, no. 77140, 5 μg/ml, both Sigma, St. Louis, MO, USA), *Mycobacterium tuberculosis* cell wall extract made of the purified filtrate of *M. tuberculosis* cultured on Sauton medium (5 μg/ml, Human, Gödöllő, Hungary), or *Candida albicans* (0656; CBS, Delft, The Netherlands). *Candida albicans* was heat-inactivated at 56°C for 30 minutes prior to the experiments and 8 *Candida*/HaCaT cell was used for stimulation.

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 pfu/cell, and cells were harvested after the indicated times. Mock-infected cells were similarly treated without addition of virus.

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

3.6 Reverse transcription polymerase chain reaction (RT-PCR)

Total RNA was isolated using Trizol reagent (Gibco BRL), according to 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 using the primers listed in Table I. Reactions contained a final concentration 0.66 pmol/μl of primer and 1.5 mM $MgCl_2$. The amplification protocol included one cycle of initial denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 2 min and one cycle of terminal extension at 72°C for 10 min. Ten microliters of PCR product was run on 1% agarose gel, stained with ethidium bromide, photographed and evaluated using a Kodak Edas 290 densitometer and Kodak 1D Digital Science software (Scientific Imaging Systems, New Haven, CT, USA).

Table I. Sequences of primers and probes used for RT-PCR and for real time RT-PCR.

	Forward primer	Reverse primer	Detection/probe
RAB10	CATGAGTTTGGAAAAAGGACAACG	CCATATCCTTTCTATGTACAATGCCG	agarose gel elecrtrophoresis
fibronectin	AAGCCAATTTCCATTAATTACCGA AC	TCTCATACTTGATGATGTAGCCGGTAA	agarose gel elecrtrophoresis
PRINS exon 2	GGCCCAGTGAGAACTACGGAA	TCATCTGAGCTTGAGTTAATCGGC	agarose gel electrophoresis
β-actin	AGAGATGGCCATGGCTGCTT	ATTTGCGGTGGACGATGGAG	agarose gel electrophoresis
PRINS exon 1	GCATCTTCCCTTGGCAAA	GCCTAAAGGACATTTCGGTAT	5'FAM TGC TGT TTT GGG TCC TAA CCA TC 3' Black Hole2
18S	CGGCTACCACATCCAAGGAA	GCTGGAATTACCGCGGCT	5'Texas Red TGC TGG CAC CAG ACT TGC CCT C 3' Black Hole2

3.7 Quantitative real time RT-PCR

For quantitative real time RT-PCR, 1 μg purified total RNA was reverse transcribed using the iScript kit (BioRad, Hercules, CA, USA). After reverse transcription, real time quantitative RT-PCR was performed to quantify the abundance of PRINS RNA. PRINS RNA expression data were normalized to the 18S ribosomal RNA expression data of each examined sample. The primers and TaqMan probes used for real time PCR are listed in Table I. The reactions were performed in duplicate, using the iQ Supermix (BioRad) in the iCycler (BioRad).

3.8 RNA interference vector construction and transfection

After analyzing the secondary structure using an RNA folding server (http://www.bioinfo.rpi.edu/applications/mfold/old/mfold), three different 19mer target sequences were selected for the sequence of PRINS. Target sequences were aligned to the human genome database in a BLAST search to ensure that the chosen sequences would work in a gene-specific manner in the subsequent siRNA experiments. Three selected siRNAs were synthesized and used in the experiments: AK696: TTTCTGGAATGATGTCCAA; AK862: TGTGGCCTGTGTTCTTTCA and AK1313: TTTTCTTTAAAGACTGCCA, where the numbers in the names of the oligos refer to the position in the transcript with GenBank accession number AK022045, which is included in the sequence of PRINS. As a control, a scrambled (nonsense) sequence of AK1313 siRNAs was used (SC1313: AACTTTATCTCGGATCTAT). All of the oligos were synthesized with *BamHI* - *HindIII*

linker sequences, according to the manual (Ambion, Inc, Austin, Texas, USA). The oligos were annealed and cloned into the *BamHI* - *HindIII* sites of pSilencer 2.1-U6 hygro as described in the manual. All the plasmids constructed were confirmed by DNA sequencing. For stable transfection of HaCaT keratinocytes as well as HeLa cells, the siRNA constructs were purified by E.Z.N.A. Plasmid Miniprep kit I (peqlab Biotechnologie GmbH, Erlangen, Germany) and 2 μg of purified plasmids were transfected to the cells using the jetPEI reagent of QBioGene (Irvine, CA, USA), following the instructions of the manual. Stable transfected cells were selected in the presence of 200 μg/ml hygromycin, several independent clones were picked up from each transformants and were further analyzed by real time RT-PCR for evaluation of the gene specific silencing of PRINS in the cells and by MTT assay to study the effect of PRINS silencing on cell viability.

3.9 MTT assay

HaCaT keratinocytes and HeLa cells were seeded in wells of a 96-well plate at a density of $3x10^3$ cells/well. Cells were either grown in serum-free media or in the presence of 10% FBS for 168h. Medium was replaced with RPMI without phenol red and 50μg of MTT (3-(4,5-dimethyl thiazol-2-yl) 2,5-diphenyl tetrazolium bromide) (Sigma) was introduced into each well and incubated for 4h at 37°C. Living cells degrade MTT by the mitochondrial succinate dehydrogenase resulting in MTT formazan. The converted dye was solubilized with acidic isopropanol (0.04M HCl in absolute isopropanol). The optical density of the wells was determined using a microplate reader (Multiscan v3.0, Labsystems) at 540nm.

3.10 DNA microarray

From HeLa cells transfected with gene-specific or control siRNA, RNA was isolated using Trizol reagent (Gibco BRL), and used for microarray experiments. For probe preparation, 2 μg of total RNA was reverse transcribed using poly-dT primed Genisphere Expression Array 900 Detection system (Genisphrere, Hatfield, PA, USA) as described (38, 39). To compare gene expression profiles of cells transfected with PRINS-specific or control siRNA, a microarray comprising 18664 human ESTs, constructed in the Biological Research

Center, Szeged, Hungary was used.

Scanned output files were analyzed using the GenePix Pro3.0 software (Axon Instruments Inc., Foster City, CA).

4. RESULTS

4.1 Differential display as well as 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. Twenty-five bands that showed consistent difference in the pair wise comparison of the samples were cut out from the acrylamide gel. The DNA content of the gels was removed by 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, reverse Southern blot technique was applied to select clones for further analysis. Fragments have been 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. Figure 2A illustrates that differential expression was confirmed for three samples (No. 1, 2 and 3), while sample No. 4 showed about the same signal intensity with the two probes, and therefore it was excluded from further analysis.

The three fragments showing elevated expression in uninvolved psoriatic epidermis (No. 1, 2 and 3) were sequenced and analyzed using the *BLASTN* program (http://www3.ncbi.nlm.nih.gov/BLAST/). Sample No. 1 was identical to the 3' end of the RAB10 gene (GenBank accession number AK023223), a member of the *RAS* oncogene family that belongs to the *rab* subfamily. Sample No. 2 was identical to the 3' end of the extracellular matrix protein fibronectin (GenBank accession number X02761) that has been demonstrated to have 24 differentially spliced variants in humans, one of which, the EDA^+ isoform was shown to be overexpressed at the dermal-epidermal junction of psoriatic uninvolved skin (20). Sample No. 3 showed 100% homology to the 3' end of a cDNA (GenBank accession number AK022045) that had been sequenced from a 10-week old human embryo cDNA library, and it had no annotated protein product and function.

4.2 The identified transcripts show differential regulation during proliferation/differentiation of keratinocytes

Since 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 (40). In this model system, serum-starved, contact-inhibited cells (0h) resemble suprabasal non-proliferating differentiated keratinocytes of normal epidermis, while the highly proliferative HaCaT cells after release from cell quiescence (24-168h) resemble the activated, differentiated, transiently amplifying keratinocytes.

RT-PCR results revealed (Fig. 2B) 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 re-addition. The highest levels of total fibronectin mRNA coincided with the highest proliferation rate of HaCaT cells (48 and 72 h). Since 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. 2B) as well as changes in the ratio of these two isoforms (19). The novel cDNA, AK022045 showed the highest level of expression in the serum starved, contact inhibited HaCaT keratinocytes (0h sample), and as the cells started to proliferate due to passaging and serum re-addition, the abundance of AK022045 cDNA dropped dramatically (24-96h samples) and did not seem to increase even after 168h, in the confluent culture (Fig. 2B).

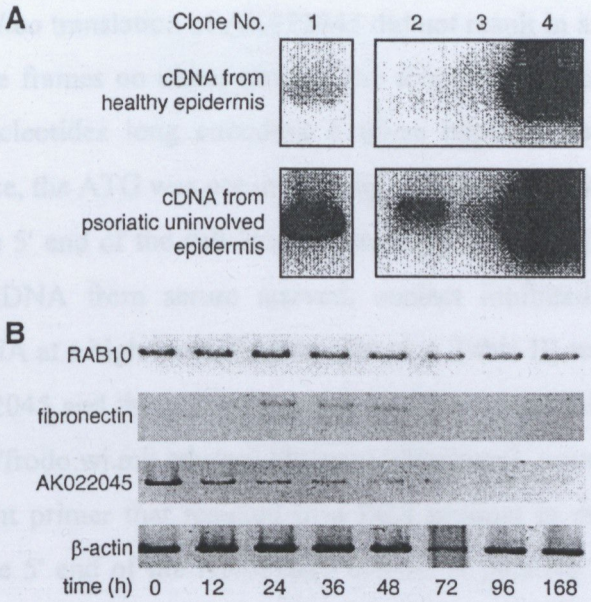

Figure 2. Verification of the differential expression of the identified transcripts in uninvolved psoriatic epidermis and their distinct regulation during keratinocyte proliferation and differentiation. *A*, Fragments identified by differential display were hybridized with radioactive labeled cDNAs from healthy and from uninvolved psoriatic epidermises (reverse Southern blotting). Differential expression was confirmed for samples No. 1, 2 and 3, while sample No. 4 showed about the same signal intensity with the two probes. *B*, The expression of RAB10, fibronectin and AK022045 transcripts (corresponding to sample no. 1, 2 and 3, respectively) was analyzed by RT-PCR in synchronized HaCaT keratinocytes after release from cell quiescence by using the primer pairs listed in Table I.

4.3 Results of the *in silico* analysis of the cDNA AK022045 are indicative for a non-coding RNA gene

To further analyze the structural characteristics of the novel cDNA, we performed *in silico* localization, structural, translation and homology studies. Results of a genomic *BLAST* search (http://www3.ncbi.nlm.nih.gov/BLAST/) showed that the genomic sequence of the transcript AK022045 was localized on the short arm of human chromosome 10 (map position: 10p12.31). Comparison of the cDNA and genomic sequences revealed that the AK022045 transcription unit is composed of two exons with an intron in between that is approximately 7

kb long. Surprisingly, *in silico* translation of AK022045 did not result in a translatable protein product in any of the three frames on either strand. The longest open reading frame on the sense strand was 180 nucleotides long encoding a 60-aa peptide; however, lacking the consensus Kozak's sequence, the ATG was not in a strong context of initiation.

In order to map the 5' end of the full-length transcript, a set of RT-PCR experiments were carried out using cDNA from serum starved, contact inhibited HaCaT cells that expressed AK022045 mRNA at a high level. Primers (listed in Table II) were designed for the cDNA sequence of AK022045 and the genomic sequence upstream of AK022045, using the Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). Figure 3A shows that the most distant primer that resulted in a PCR product in our experiments was 1483 bp upstream from the 5' end of the AK022045 cDNA. A putative TFIIB transcription factor binding site has also been identified on the genomic sequence proximal from the possible start of transcription.

Further homology and similarity searches revealed that the full-length cDNA identified in HaCaT keratinocytes harbors 2 *Alu* elements and a short sequence element that shows approximately 70% similarity with an element in a small cytoplasmic RNA (G8 RNA) responsible for developing thermotolerance in *Tetrahymena thermophila* (Fig. 3B). These characteristics and the fact that the transcript could not be translated *in silico* into any protein sequence prompted us to hypothesize that AK022045 is a non-coding RNA gene. Since 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 *P*soriasis *S*usceptibility Related *R*NA gene *In*duced by *S*tress (PRINS).

Table II. Sequence of primers used for RT-PCR for the mapping of PRINS 5' end.

Position* and sequence of forward primers	Position and sequence of reverse primers	Expected length of amplicon (bp)
-554 TTTAGTAAACAATCTACCGAGCAGT	+302 AAAACAAATGGTGGGCTGAG	846
-1141 TGCTGCAGATAATCGTTTGG	-370 CCAGCAAACCCAGCATAAGT	771
-1483 CCTTTTCCTTTCCGAGACAA	-1118 CAGGCCAAACGATTATCTGC	365
-2369 ACAGGGTTTTGCCGTGTTAC	-1464 TTGTCTCGGAAAGGAAAAGGA	905

*Relative to the first nucleotide in the sequence of the cDNA with accession number AK022045

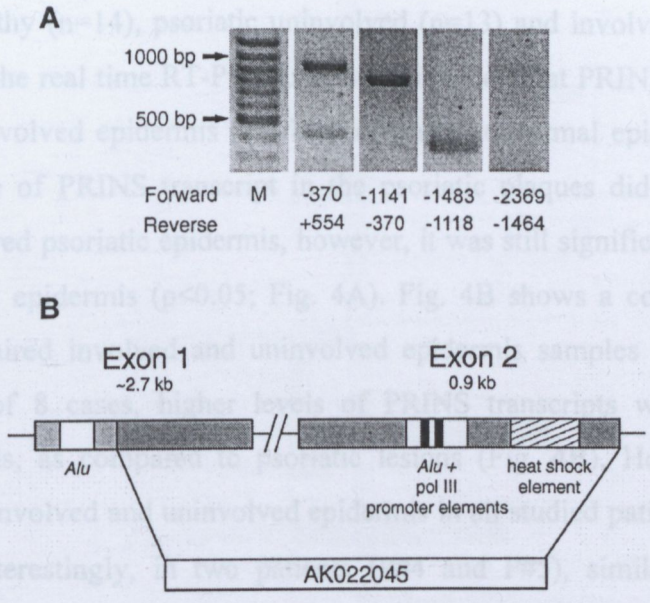

Figure 3. The structure of the full-length PRINS transcript in HaCaT keratinocytes. *A*, Total RNA was isolated from HaCaT keratinocytes, reverse transcribed and RT-PCR reactions were carried out using the primer pairs listed in Table II. Primers were designed for the already known sequence of the transcript with GenBank accession number AK022045 and for the genomic sequence proximal to its 5' end (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 1483bp but no more than 2369bp longer than the originally sequenced cDNA, AK022045. *B*, Comparison of the cDNA and genomic sequences revealed that the AK022045 transcript consists of 2 exons. Homology searches revealed the presence of 2 *Alu* elements within the PRINS sequence and a sequence that showed approximately 70% similarity with an element necessary for the thermotolerance function of a small cytoplasmic RNA (G8 RNA) in *Tetrahymena thermophila*.

4.4 PRINS is overexpressed in uninvolved psoriatic skin and its expression is regulated by T lymphokines

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 to normal epidermis, $p<0.01$; Fig. 4A). 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 to healthy epidermis ($p<0.05$; Fig. 4A). Fig. 4B shows a comparison of PRINS expression in the paired involved and uninvolved epidermis samples from eight psoriatic patients. In 6 out of 8 cases, higher levels of PRINS transcripts were detected in the uninvolved epidermis, as compared to psoriatic lesions (Fig. 4B). However, PRINS was upregulated in both involved and uninvolved epidermis in all studied patients, as compared to 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. 4B).

To obtain further insights into the regulation of PRINS in the skin, we studied the expression of PRINS in organotypic cultures by real time RT-PCR. Healthy (n=5) and psoriatic uninvolved (n=5) epidermis specimen were treated with T-cell lymphokines, either with interferon-γ (IFN-γ) or the mixture of IFN-γ, interleukin-3 (IL-3) and granolucyte macrophage colony-stimulating factor (GM-CSF), the latter combination known to induce keratinocyte hyperproliferation in uninvolved psoriatic, but not in normal keratinocytes *in vitro* (17). 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 to the untreated uninvolved epidermis (Fig. 4C). IFN-γ treatment alone did not change PRINS expression in the uninvolved epidermis (Fig. 4C). 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.

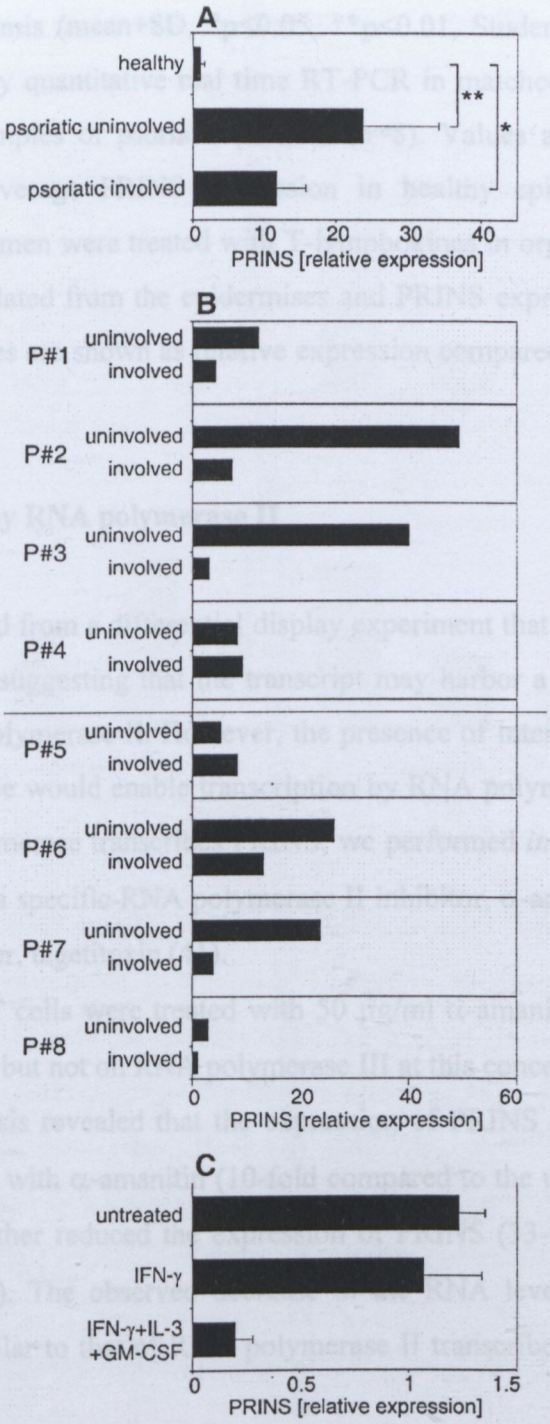

Figure 4. PRINS is overexpressed in the uninvolved epidermis of psoriatic patients and T-lymphokines decrease its expression. *A*, PRINS expression was analyzed by quantitative real time RT-PCR in healthy (n=14), psoriatic uninvolved (n=13) and involved (n=14) epidermis samples. Primers listed in Table I were used. Values are shown as relative expression

compared to healthy epidermis (mean+SD, *p<0.05, **p<0.01, Student's *t*-test). *B*, PRINS expression was compared by quantitative real time RT-PCR in matched pairs of uninvolved and involved epidermis samples of psoriatic patients (n=8). Values are shown as relative expression compared to average PRINS expression in healthy epidermis. *C*, Psoriatic uninvolved (n=5) skin specimen were treated with T-lymphokines in organotypic cultures for 3 days. Total RNA was isolated from the epidermises and PRINS expression was compared by real time RT-PCR. Values are shown as relative expression compared to untreated healthy epidermis (mean+SE).

4.5 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 polyA tail indicative for transcription by RNA polymerase II. However, the presence of internal RNA polymerase III promoters in the sequence would enable transcription by RNA polymerase III. In order 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 (41).

Subconfluent HaCaT cells were treated with 50 μg/ml α-amanitin that has an effect only on RNA polymerase II but not on RNA polymerase III at this concentration (42). Results of real time RT-PCR analysis revealed that the expression of PRINS dropped dramatically after a 5-hour co-incubation with α-amanitin (10-fold compared to the untreated control) and incubation for 25 hours further reduced the expression of PRINS (33-fold compared to the untreated control) (Fig. 5A). The observed decrease in the RNA level of PRINS after α-amanitin treatment was similar to that of RNA polymerase II transcribed genes such as IL-8 (Fig. 5B).

In contrast to α-amanitin, tagetitoxin (1 μM and 3 μM for 6 and 12 hours) did not change PRINS expression significantly compared to the untreated controls (Fig 5C). 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 *7SL* gene at both

concentrations, indicating that RNA polymerase III was effectively inhibited by these concentrations of tagetitoxin (Fig. 5D).

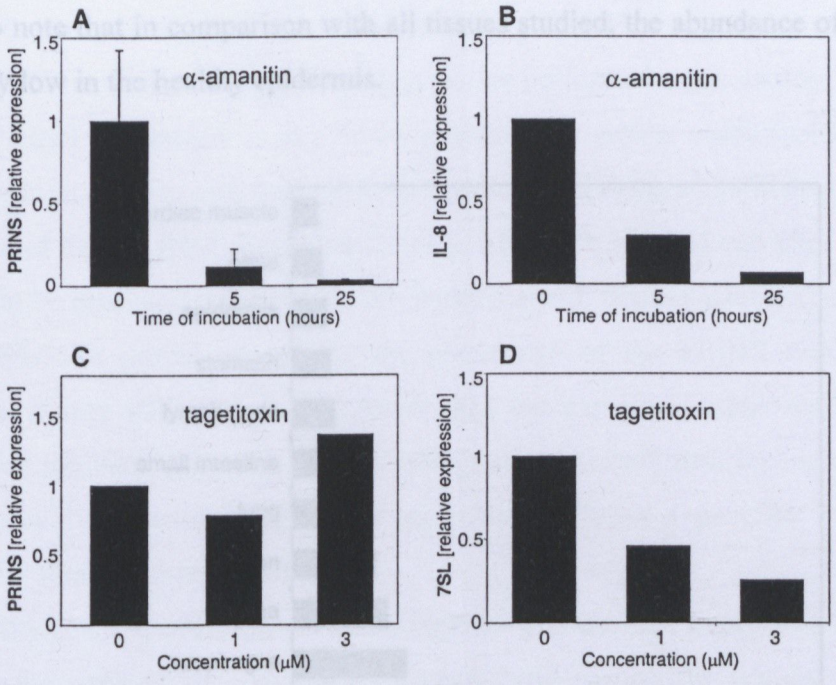

Figure 5. PRINS is transcribed by RNA polymerase II. HaCaT keratinocytes were co-incubated either with the specific RNA polymerase II inhibitor, α-amanitin (20 μg/ml) for 5 and for 25 hours (n=3) (*A* ,*B*) or with the specific RNA polymerase III inhibitor, tagetitoxin (1 μM and 3 μM) for 12 hours (n=2) (*C, D*). Quantitative real time PCR analysis of PRINS (*A, C*), the RNA polymerase II transcribed IL-8 (*B*) and *7SL,* a well-characterized RNA polymerase III transcribed gene (*D*).Values are indicated as relative expression compared to the untreated controls. Error bars show SD of three independent experiments.

4.6 PRINS is expressed in various human tissues and the level of expression shows large differences

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. 6). Interestingly, the level of expression differed to a great extent in different organs. The lowest

level of PRINS RNA was detected in cardiac muscle, while 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 in comparison with all tissues studied, the abundance of the transcript was relatively low in the healthy epidermis.

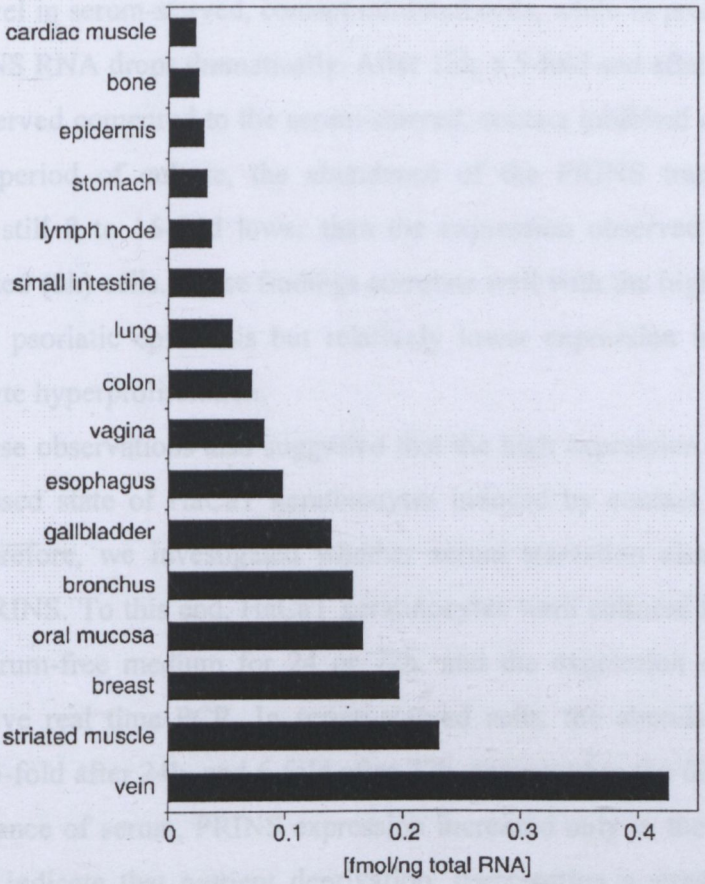

Figure 6. PRINS is expressed in various human organs. Total RNA was isolated from tissue samples from various human organs and PRINS RNA abundance was detected by quantitative real time RT-PCR. Data are expressed as fmol of PRINS in 1 ng of total RNA. Mean values of three to five independent samples are shown.

4.7 PRINS is induced by environmental stress factors

4.7.1 PRINS is induced by serum starvation and contact inhibition

In our initial experiments, using the model system of synchronized HaCaT

keratinocytes, the AK022045 cDNA was abundantly expressed in contact-inhibited, non-proliferating, differentiated cells while its expression decreased in proliferating cells (Fig. 2B). To further confirm 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, while in proliferating cells, the abundance of PRINS RNA drops dramatically. After 12h, a 5-fold and after 24h, a 50-fold decrease could be observed compared to the serum-starved, contact inhibited cells (Fig. 7A). During the 36-168h period of culture, the abundance of the PRINS transcript slightly increased, but it was still 8 to 16-fold lower than the expression observed in the serum-starved, contact inhibited (0h) 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 72h, 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 24h, and 6-fold after 72h, compared to the 0h controls (Fig. 7B), while in the presence of serum, PRINS expression increased only in the 72h confluent culture. These results indicate that nutrient deprivation, representing a stress situation for keratinocytes, induces the expression of PRINS.

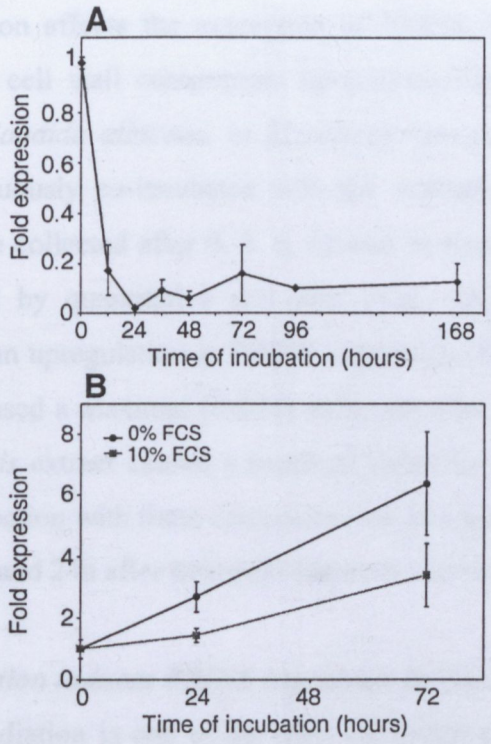

Figure 7. PRINS expression is dependent on the proliferation/differentiation of keratinocytes and it is induced by serum starvation. *A*, HaCaT keratinocytes were synchronized by serum starvation and contact inhibition, and after release from cell quiescence, samples were collected at the indicated time points. PRINS expression was determined by quantitative real time RT-PCR. Data are indicated as fold expression compared to the serum-starved, contact inhibited (0h) samples. Values represent means of three independent experiments±SE. *B*, Subconfluent HaCaT keratinocytes were cultured either under normal serum conditions (10% FCS) or in serum-free medium (0% FCS) for 24 or 72 hours. PRINS expression was determined by quantitative real time RT-PCR. Data are indicated as fold expression compared to the initial, 0h samples. Values represent means of three independent experiments±SE.

4.7.2 Microbial compounds and Candida albicans 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. Epidermal keratinocytes, representing a barrier between the human body and the environment, are often exposed to various infectious agents such as bacteria and fungi. To determine whether

bacterial or fungal infection affects the expression of PRINS, HaCaT keratinocytes were treated with the bacterial cell wall components lipopolysaccharide (LPS), peptidoglycane (PGN), heat-inactivated *Candida albicans,* or *Mycobacterium tuberculosis* extract. HaCaT keratinocytes were continuously co-incubated with the microbial compounds or *Candida albicans* and samples were collected after 0, 3, 6, 12 and 24 hours. The level of the PRINS transcript was determined by quantitative real time PCR. All the microbial and fungal compounds tested caused an upregulation in PRINS expression (Fig. 8A, $p<0.05$). Treatment with *Candida albicans* caused a maximal (8-fold) induction after 24h, while LPS, PGN and *Mycobacterium tuberculosis* extract caused a maximal induction of PRINS after 12h (10 to 14-fold increase). Co-incubation with these compounds for 1h caused only a slight increase in PRINS expression 3, 6, 12 and 24h after treatment (data not shown).

4.7.3 Ultraviolet B irradiation induces PRINS expression in HaCaT keratinocytes

Ultraviolet (UV) radiation is one of the most important environmental stress factor, 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 crosslinking and modifying the pattern of gene expression (43). The effect of UV-B exposure (110 mJ/cm^2) on PRINS expression was examined 3, 6, 12 and 24 hours after the irradiation of HaCaT cells. Results of quantitative real time PCR analysis showed that UV-B irradiation significantly induced PRINS expression already after 3h ($p<0.05$; Fig. 8B), and peak expression was observed 24h after irradiation.

4.7.4 Viral infection as well as inhibition of translation upregulates PRINS expression

To investigate the effect of viral infection on PRINS expression, HaCaT cells were infected with herpes simplex virus type 1 (HSV-1) at a multiplicity of infection of 0.01 pfu/cell. The abundance of PRINS transcript was determined by quantitative real time PCR 0, 3, 6, 12 and 24 hours after infection. Compared to the time-matched mock-infected control, HSV-1 infection caused a 2.5-fold upregulation in PRINS expression already after 3h (Fig. 8C, $p<0.05$). The abundance of PRINS RNA dropped back after 12h, but after 24h a second increase was observed.

Viral infection causes complex changes in host cell biosynthesis, including inhibition of translation of selective host mRNAs (44). In order to investigate whether direct

SZTE Egyetemi Könyvtár SZEGED 14.

translational inhibition affects the expression of PRINS, HaCaT keratinocytes were treated with cycloheximide, an inhibitor of protein synthesis, at 20 μg/ml final concentration, and the abundance of PRINS RNA was determined by quantitative real time PCR. PRINS expression was rapidly induced by cycloheximide treatment, already after 30 minutes ($p<0.01$; Fig. 8D), and it was still elevated after 12 hours (Fig. 8D).

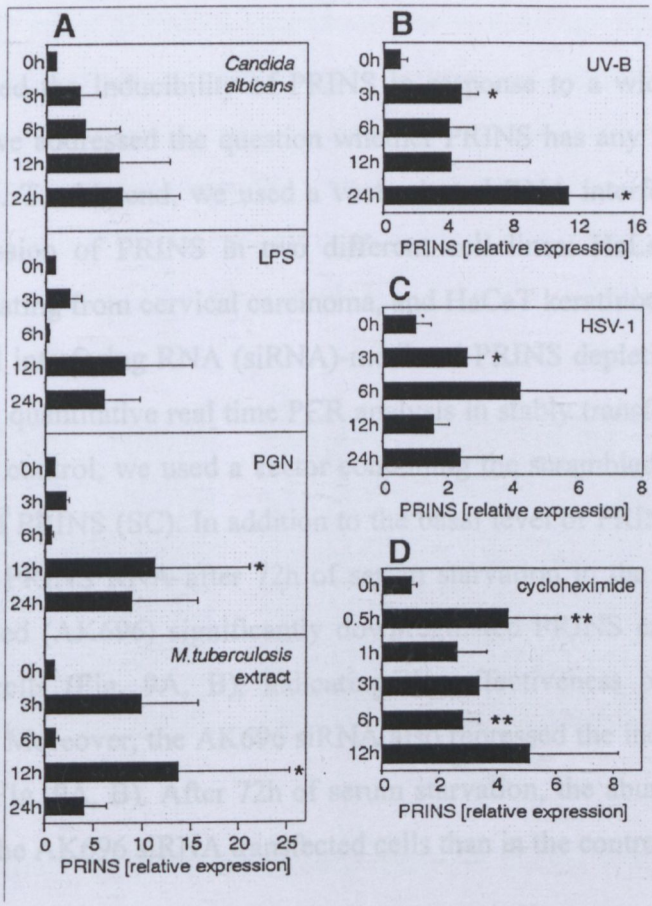

Figure 8. Environmental stress signals induce the expression of PRINS. *A*, HaCaT keratinocytes were co-incubated with heat-inactivated *Candida albicans*, lipopolysaccharide (LPS), peptidoglycane (PGN) or *Mycobacterium tuberculosis* extract for the indicated times. *B*, HaCaT keratinocytes were exposed to UV-B irradiation (110 mJ/cm^2), and samples were collected at the indicated time points after irradiation. *C*, HaCaT keratinocytes were infected with herpes simplex virus type 1 (HSV-1) at a multiplicity of infection of 0.01 pfu/cell and samples were collected at the indicated times after virus adsorption. *D*, The translational inhibitor cycloheximide (20 μg/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 to the time-matched untreated controls. Values represent means of ≥4 independent experiments±SE (*$p<0.05$, **$p<0.01$, Student's *t*-test).

4.8 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 whether PRINS has any functional role in cellular stress responses. To this end, we used a vector-based RNA interference method to knock down the expression of PRINS in two different cell lines: HeLa cells, a human epithelial cell line originating from cervical carcinoma, and HaCaT keratinocytes. To evaluate the efficacy of the small interfering RNA (siRNA)-mediated PRINS depletion, PRINS RNA level was determined by quantitative real time PCR analysis in stably transfected HaCaT and HeLa cells. As negative control, we used a vector containing the scrambled sequence of one of the studied siRNAs of PRINS (SC). In addition to the basal level of PRINS RNA, we also determined the level of PRINS RNA after 72h of serum starvation in the transfected cells. One of the siRNAs tested (AK696) significantly downregulated PRINS expression both in HeLa and in HaCaT cells (Fig. 9A, B), indicating the effectiveness of this siRNA in degrading PRINS RNA. Moreover, the AK696 siRNA also repressed the induction of PRINS in serum-starved cells (Fig. 9A, B). After 72h of serum starvation, the abundance of PRINS RNA was still lower in the AK696 siRNA transfected cells than in the controls.

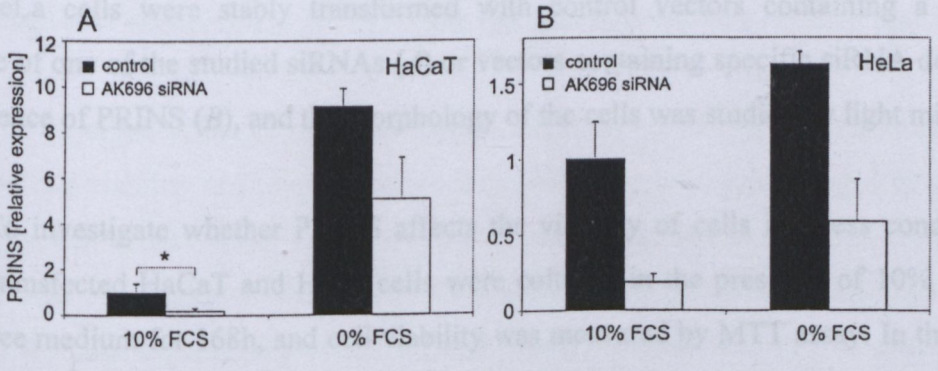

Figure 9. Gene-specific silencing by siRNA effectively downregulates PRINS. HaCaT keratinocytes *(A)* and HeLa cells *(B)* transfected with the pSilencer vector containing siRNA

specific for PRINS (*AK696*) or a scrambled sequence (control) were cultured in medium containing 10% or 0% FCS for 72h. PRINS expression was determined by quantitative real time PCR. Data are indicated as fold expression compared to the controls cultured in the presence of 10% FCS. Values represent means of at least two independent experiments+SD (*$p<0.05$, Student's *t*-test).

To investigate the effect of PRINS knockdown, the morphology of stably transfected cells was examined by light microscopy. Interestingly, HeLa cells transfected with the siRNA *AK696* showed a different morphology compared to both untransfected HeLa cells and to those transfected with the vector containing the scrambled sequence (HeLa/*control*) (Fig. 10). *AK696*-transfected HeLa cells (Fig. 10A) showed a more elongated, spindle-like morphology and formed less colonies as compared to the control HeLa cells (Fig. 10B).

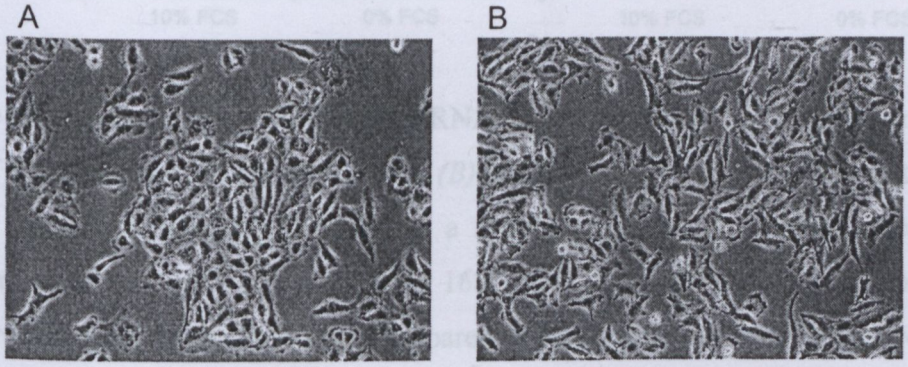

Figure 10. PRINS depletion by RNA interference results in altered morphology in HeLa cells. HeLa cells were stably transformed with control vectors containing a scrambled sequence of one of the studied siRNAs (*A*) or vectors containing specific siRNA designed for the sequence of PRINS (*B*), and the morphology of the cells was studied by light microscopy.

To investigate whether PRINS affects the viability of cells in stress conditions, the siRNA-transfected HaCaT and HeLa cells were cultured in the presence of 10% FCS or in serum-free medium for 168h, and cell viability was measured by MTT assay. In the presence of serum, cell viability was not significantly affected by the downregulation of PRINS, neither in HaCaT keratinocytes (Fig. 11A) nor in HeLa cells (Fig. 11B). However, after 168h,

cell viability was significantly decreased in the serum-starved *AK696* siRNA-transfected cells as compared to the controls containing the scrambled siRNA ($p<0.001$, both in HaCaT and HeLa cells) as well as compared to the *AK696* siRNA containing cells cultured in the presence of serum ($p<0.001$ in HaCaT keratinocytes, $p<0.05$ in HeLa cells, Fig. 11A, B). These findings demonstrate that the downregulation of PRINS impairs cell viability in serum-starved cells, suggesting that PRINS is essential for cell survival under stress conditions.

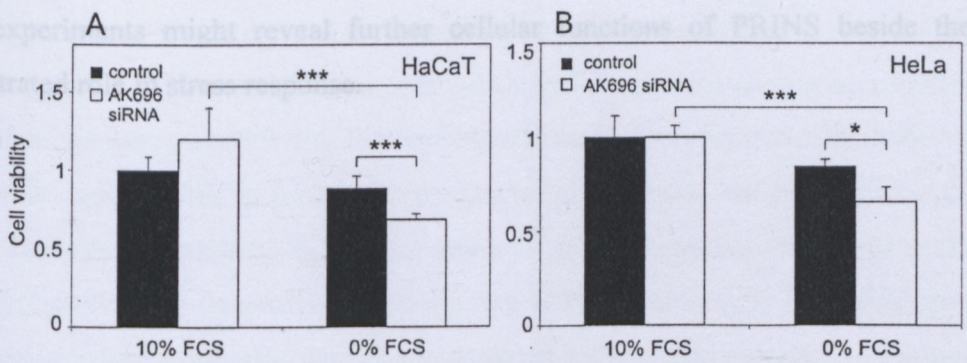

Figure 11. Gene-specific silencing by siRNA decreases cell viability in serum-starved cells. HaCaT keratinocytes *(A)* and HeLa cells *(B)* transfected with the pSilencer vector containing siRNA specific for PRINS (*AK696*) or a scrambled sequence (control) were cultured in medium containing 10% or 0% FCS for 168h. Cell viability was measured by MTT assay. Data are indicated as relative values compared to the controls cultured in the presence of 10% FCS. Values represent means of at least six independent experiments+SD (*$p<0.05$, ***$p<0.001$, Student's *t*-test).

4.9 Gene-specific silencing of PRINS affects the gene expression profile of HeLa cells

The observed effect of the gene-specific silencing of PRINS on cell morphology as well as on cell viability in stress conditions prompted us to hypothesize that PRINS exerts a specific function in the cells as a regulatory RNA, modifying the expression of protein-coding genes. In order to investigate whether PRINS regulates the expression of other transcripts, we compared the gene expression profiles in HeLa cells transfected with PRINS-specific siRNA (*AK696*) or with a scrambled sequence (control). For this, we used an oligonucleotide array comprising 18664 ESTs to initiate a comprehensive analysis of the transcriptional changes

that occur when PRINS is downregulated. 66 transcripts were differentially expressed in the PRINS siRNA transfected cells compared to controls: 40 genes with known function and 26 transcripts with unknown function. Among the differentially expressed transcripts, 24 were downregulated and 42 were upregulated in the PRINS siRNA transfectants. Among the regulated transcripts, several encode proteins that are involved in cell cycle regulation, immunological processes and cell morphology. Current investigations focus on the analysis of the data and the validation of the gene expression differences by quantitative real time PCR. These experiments might reveal further cellular functions of PRINS beside the already demonstrated role in stress response.

5. DISCUSSION

Psoriasis is a chronic inflammatory skin disease with multifactorial origin. Genetic, immunological and environmental factors play a role in the precipitation of the psoriatic symptoms, however, the interplay of these factors in the pathogenesis of psoriasis is still poorly understood (1, 7). There is evidence that the healthy-appearing, uninvolved epidermis of psoriatic patients differs from the epidermis of the healthy individuals in its response to physical stress and T cell derived cytokines as well as in its molecular profile (16, 17, 21). Indentification of differentially expressed genes in the uninvolved psoriatic epidermis may reveal novel genetic susceptibility factors responsible for the elevated sensitivity of psoriatic uninvolved keratinocytes to proliferative signals. In this work, we performed a differential display analysis, a large-scale, "open system" gene expression study comparing gene expression profiles of uninvolved psoriatic and healthy epidermis. The advantage of such "open system" gene expression studies as compared with "closed systems" is that not only the expression of already annotated genes can be compared, but yet uncharacterized transcripts can also be identified. Characterization of differentially expressed, yet unknown transcipts may reveal novel pathways involved in psoriasis susceptibility and may provide new insights into the pathogenesis of this complex disease.

Using differential display analysis comparing gene expression profiles of uninvolved psoriatic and healthy epidermis, 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 (19). 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 (18, 20). The fact that using differential display we could also detect abnormal fibronectin mRNA expression in psoriatic uninvolved epidermis confirmed that the experimental setup we applied was an appropriate tool for identifying gene expression differences in psoriatic uninvolved epidermis. Another transcript showing elevated expression in psoriatic uninvolved epidermis was RAB10, a member of the *ras* oncogene family. The RAB10 protein plays a role in vesicular transport (45). Elevated RAB10 gene expression has also been found previously in other pathological conditions such as hepatocellular carcinoma (45) and melanoma (46).

Beside genes with already known and characterized functions, we also identified a yet uncharacterized transcript showing elevated level in psoriatic uninvolved epidermis. 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 non-coding RNA. Homology studies on the full-length cDNA sequence also supported this hypothesis, since the sequence contains an element with high-level homology to a heat shock element in a small noncoding RNA, *G8* (47). Moreover, it also harbors *Alu* repetitive elements, similarly to already characterized non-coding RNA genes such as the neuronspecifically expressed non-coding human RNA gene, *BC200* (42). Based on its high expression in psoriatic uninvolved epidermis and the observed stress-inducibility in *in vitro* experiments, we named the novel transcript PRINS (*P*soriasis-susceptibility Related *R*NA Gene *In*duced by *S*tress). 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 non-coding 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 ORF (48-50).

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 (51, 52), and it has been shown that microvascular changes occur early in the development of psoriatic lesions (51). 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 to healthy epidermis, but lower than in the uninvolved epidermis. Moreover, lymphokine treatment, known to induce hyperproliferation of uninvolved psoriatic keratinocytes, decreased 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 (17, 19, 20). 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. To date, no genetic loci associated with psoriasis susceptibility have been mapped on human chromosome 10p, where PRINS is coded. PRINS might contribute to psoriasis susceptibility as a minor modifier gene beside the effect of the classical PSORS 1-9 loci at chromosomes 19p13, 17q25, 1q21, 1p, 6p21.3, 4q31-q34, 4q, 3q21 (1, 15). 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 heterogenity 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, non-proliferating 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 upregulation 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(47, 53), prompted us to hypothesize that PRINS is induced also by other types of stress stimuli. Indeed, skin-relevant environmental stresses as divergent as UV-B irradiation, viral infection (HSV-1), various microbial compounds (LPS, peptidoglycane, *Mycobacterium tuberculosis* extract) and fungi (*Candida albicans*) increased the RNA level of PRINS in HaCaT keratinocytes. In most cases, peak expression was observed 12-24 hours after stimulation, indicating that PRINS may not be induced directly by the different stimuli, but its induction may rather be a late step in the integrated network of intracellular signaling induced by environmental stress signals. Bacterial, fungal and possibly also viral components can be recognized by Toll-like receptors in keratinocytes, and induce signal transduction pathways leading to the production of chemotactic and inflammatory mediators (54, 55). It is conceivable that the complex network of pathways induced by these environmental stress factors include a common stress response pathway, involving the upregulation of PRINS. In addition to skin-relevant stress signals, 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 (56-58) and translation inhibition (32, 53, 56). 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.

In addition to *Alu* elements, several non-coding 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 *Tetrahymena thermophila* (47), the *gadd7* RNA is induced by DNA damaging agents (59), while *adapt15* and *adapt33* RNAs are induced in response to oxidative stress (60, 61). However, how these RNAs exert their function under stress conditions is not entirely understood. The so far identified non-coding RNAs can have diverse regulatory functions such as the regulation of chromatin modification, transcription, alternative splicing, mRNA stability, translation or cell

signaling. *BC200*, a small RNA expressed in neurons plays a role in the posttranscriptional regulation of several proteins at the post-synaptic regions of dendrits (42, 62). The *B2* gene, harboring the mouse orthologue of human *Alu* elements, represses mRNA expression in cells exposed to heat shock, thus functions as a transcriptional regulator (62, 63).

To explore whether PRINS plays a functional role in the stress response of keratinocytes, we used RNA interference to knock down PRINS expression in HaCaT keratinocytes and in HeLa cells. Downregulation 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 cells exposed to stress and might have a protective function against stress-induced cell death. Interestingly, knockdown of PRINS expression also resulted in morphological changes in HeLa cells. Since PRINS siRNA-transfected HeLa cells formed less and smaller colonies, it is conceivable that PRINS may also regulate genes involved in cell adhesion and cell-cell connections.

To determine whether PRINS regulates the expression of other genes at the transcriptional level, gene expression profiles of PRINS siRNA-transfected and control HeLa cells were compared using DNA microarray. Several gene expression differences were found, indicating that PRINS may regulate the expression of protein-coding genes at the transcriptional level. Currently ongoing investigations focus on the validation of the gene expression differences by quantitative real time RT-PCR. Identification of genes regulated by PRINS may reveal novel factors related to psoriasis and may help to enhance our understanding about the pathomechanism of this disease.

Several non-coding transcripts have been shown to be abnormally expressed in various human diseases, which emphasizes the importance of understanding their functions in normal cells. *BC200*, a small, normally neuronspecifically expressed RNA gene harboring a monomeric *Alu* element, is also expressed in several human tumor types (64). A recent study has shown that *BC200* is expressed at high levels in invasive carcinomas of the breast, but it is not detectable in normal breast tissue or in benign tumors such as fibroadenomas, demonstrating the potential of *BC200* expression to serve as a molecular tool in the diagnosis of breast cancer (65). *MALAT-1* is overexpressed in non-small cell lung cancer (NSCLC), has a stable non-coding RNA product of 8 kb length, and the elevated level of *MALAT-1* expression was significantly associated with metastasis in NSCLC patients (66). Another non-coding RNA gene (GenBank Acc. No.: AY166681), recently identified from the NSCLC-N6

cell line has a role in the proliferation arrest induced by VT1, a promising anti-tumor drug (67). A prostate-specific non-coding RNA gene, *PCGEM1* shows a significant overexpression in 84% of the prostate cancer patients and it is expressed in an androgen-dependent manner (50). It has been recently demonstrated that *PCGEM1* overexpression in NIH3T3 cells promotes proliferation and a dramatic increase in colony formation (68). Taken together, growing evidence suggests that several non-coding RNA genes show abnormal expression in diseased tissues, and this abnormal expression affects the proliferation of the cells. Thus, non-coding RNAs may play an important role in the pathomechanism of common diseases, in particular disorders associated with abnormal cell proliferation, such as cancer or psoriasis. Hence, these transcripts could serve as potential molecular markers or novel targets for future drug designs.

Our initial results indicating that PRINS depletion affects the gene expression profile suggest that PRINS may function as a "riboregulator", modifying the expression of other genes. Further studies of this non-coding RNA will provide new insights into the complex stress response pathways as well as into the role of non-coding RNAs in the pathogenesis of human diseases. Moreover, identification and characterization of genes regulated by PRINS may reveal novel factors associated with psoriasis and enhance our understanding about the pathophysiology of this complex disease.

6. SUMMARY

- Using differential display analysis comparing gene expression profiles of psoriatic uninvolved and healthy epidermis, we have identified several transcripts which may play a role in psoriasis susceptibility.
- We further studied one of the yet uncharacterized transcripts showing elevated expression in uninvolved epidermis, which we named PRINS (*P*soriasis-susceptibility Related *R*NA Gene *In*duced by *S*tress). Using *in silico* analysis, we showed that PRINS has no translatable protein product but harbours elements homologous to non-coding RNA genes, suggesting that PRINS functions as a non-coding RNA.
- We have demonstrated that the PRINS transcript expressed in HaCaT keratinocytes is transcribed by RNA polymerase II and that PRINS is widely expressed in various human tissues.
- We have confirmed using real time RT-PCR analysis on several independent samples that PRINS is higher expressed in the uninvolved epidermis of psoriatic patients compared to both psoriatic lesional and healthy epidermis, suggesting a role for PRINS in psoriasis susceptibility.
- We have demonstrated that PRINS is regulated by the proliferation and differentiation state of keratinocytes.
- We have shown that environmental stress signals as divergent as serum starvation, UV-B irradiation, viral infection and various microbial compounds increase the RNA level of PRINS, indicating that PRINS is a stress-inducible gene.
- Gene-specific silencing of PRINS by RNA interference revealed that downregulation of PRINS impairs cell viability after serum starvation but not under normal serum conditions, suggesting a protective role for PRINS under stress conditions.
- We have shown that silencing of PRINS in HeLa cells results in altered cell morphology and causes changes in the gene expression profile, indicating that PRINS exerts its function by regulating the expression of other genes.

7. REFERENCES

1. Nickoloff, B. J., and F. O. Nestle. 2004. Recent insights into the immunopathogenesis of psoriasis provide new therapeutic opportunities. *J. Clin. Invest. 113:1664.*
2. Finlay, A. Y., G. K. Khan, D. K. Luscombe, and M. S. Salek. 1990. Validation of Sickness Impact Profile and Psoriasis Disability Index in Psoriasis. *Br J Dermatol 123:751.*
3. Bowcock, A. M., W. Shannon, F. Du, J. Duncan, K. Cao, K. Aftergut, J. Catier, M. A. Fernandez-Vina, and A. Menter. 2001. Insights into psoriasis and other inflammatory diseases from large-scale gene expression studies. *Hum. Mol. Genet. 10:1793.*
4. Menter, A., and J. N. Barker. 1991. Psoriasis in practice. *Lancet 338:231.*
5. Gudjonsson, J. E., A. Johnston, H. Sigmundsdottir, and H. Valdimarsson. 2004. Immunopathogenic mechanisms in psoriasis. *Clin Exp Immunol 135:1.*
6. Baker, B. S., S. Bokth, A. Powles, J. J. Garioch, H. Lewis, H. Valdimarsson, and L. Fry. 1993. Group A streptococcal antigen-specific T lymphocytes in guttate psoriatic lesions. *Br J Dermatol 128:493.*
7. Krueger, J. G., and A. Bowcock. 2005. Psoriasis pathophysiology: current concepts of pathogenesis. *Ann Rheum Dis 64:ii30.*
8. Bowcock, A. M., and J. N. Barker. 2003. Genetics of psoriasis: the potential impact on new therapies. *J Am Acad Dermatol 49:S51.*
9. Bhalerao, J., and A. Bowcock. 1998. The genetics of psoriasis: a complex disorder of the skin and immune system. *Hum. Mol. Genet. 7:1537.*
10. Helms, C., L. Cao, J. G. Krueger, E. M. Wijsman, F. Chamian, D. Gordon, M. Heffernan, J. A. Daw, J. Robarge, J. Ott, P. Y. Kwok, A. Menter, and A. M. Bowcock. 2003. A putative RUNX1 binding site variant between SLC9A3R1 and NAT9 is associated with susceptibility to psoriasis. *Nat Genet 35:349.*
11. Holm, S. J., L. M. Carlen, L. Mallbris, M. Stahle-Backdahl, and K. P. O'Brien. 2003. Polymorphisms in the SEEK1 and SPR1 genes on 6p21.3 associate with psoriasis in the Swedish population. *Exp Dermatol 12:435.*
12. Orru, S., E. Giuressi, M. Casula, A. Loizedda, R. Murru, M. Mulargia, M. V. Masala, D. Cerimele, M. Zucca, N. Aste, P. Biggio, C. Carcassi, and L. Contu. 2002. Psoriasis is associated with a SNP haplotype of the corneodesmosin gene (CDSN). *Tissue Antigens 60:292.*
13. Sagoo, G. S., R. Tazi-Ahnini, J. W. N. Barker, J. T. Elder, R. P. Nair, L. Samuelsson, H. Traupe, R. C. Trembath, D. A. Robinson, and M. M. Iles. 2004. Meta-Analysis of Genome-Wide Studies of Psoriasis Susceptibility Reveals Linkage to Chromosomes 6p21 and 4q28-q31 in Caucasian and Chinese Hans Population. *J Invest Dermatol 122:1401.*
14. Elder, J. T. 2005. Fine mapping of the psoriasis susceptibility gene PSORS1: a reassessment of risk associated with a putative risk haplotype lacking HLA-Cw6. *J Invest Dermatol 124:921.*
15. Bowcock, A. M. 2004. Psoriasis Genetics: The Way Forward. *J Invest Dermatol 122:xv.*
16. Hatta, N., M. Takata, S. Kawara, T. Hirone, and K. Takehara. 1997. Tape stripping induces marked epidermal proliferation and altered TGF-alpha expression in non-lesional psoriatic skin. *J Dermatol Sci 14:154.*

17. Bata-Csorgo, Z., C. Hammerberg, J. J. Voorhees, and K. D. Cooper. 1995. Kinetics and regulation of human keratinocyte stem cell growth in short-term primary ex vivo culture. Cooperative growth factors from psoriatic lesional T lymphocytes stimulate proliferation among psoriatic uninvolved, but not normal, stem keratinocytes. *J Clin Invest 95:317.*

18. Bata-Csorgo, Z., K. D. Cooper, K. M. Ting, J. J. Voorhees, and C. Hammerberg. 1998. Fibronectin and alpha5 integrin regulate keratinocyte cell cycling. A mechanism for increased fibronectin potentiation of T cell lymphokine-driven keratinocyte hyperproliferation in psoriasis. *J Clin Invest 101:1509.*

19. Szell, M., Z. Bata-Csorgo, A. Koreck, A. Pivarcsi, H. Polyanka, C. Szeg, M. Gaal, A. Dobozy, and L. Kemeny. 2004. Proliferating keratinocytes are putative sources of the psoriasis susceptibility-related EDA+ (extra domain A of fibronectin) oncofetal fibronectin. *J Invest Dermatol 123:537.*

20. Ting, K. M., D. Rothaupt, T. S. McCormick, C. Hammerberg, G. Chen, A. C. Gilliam, S. Stevens, L. Culp, and K. D. Cooper. 2000. Overexpression of the oncofetal Fn variant containing the EDA splice-in segment in the dermal-epidermal junction of psoriatic uninvolved skin. *J Invest Dermatol 114:706.*

21. Zhou, X., J. G. Krueger, M.-C. J. Kao, E. Lee, F. Du, A. Menter, W. H. Wong, and A. M. Bowcock. 2003. Novel mechanisms of T-cell and dendritic cell activation revealed by profiling of psoriasis on the 63,100-element oligonucleotide array. *Physiol. Genomics 13:69.*

22. Marshall, A., and J. Hodgson. 1998. DNA chips: an array of possibilities. *Nat Biotechnol 16:27.*

23. Green, C. D., J. F. Simons, B. E. Taillon, and D. A. Lewin. 2001. Open systems: panoramic views of gene expression. *J Immunol Methods 250:67.*

24. Nomura, I., B. Gao, M. Boguniewicz, M. A. Darst, J. B. Travers, and D. Y. Leung. 2003. Distinct patterns of gene expression in the skin lesions of atopic dermatitis and psoriasis: a gene microarray analysis. *J Allergy Clin Immunol 112:1195.*

25. Abts, H. F., T. Welss, A. Mirmohammadsadegh, K. Kohrer, G. Michel, and T. Ruzicka. 1999. Cloning and characterization of hurpin (protease inhibitor 13): A new skin-specific, UV-repressible serine proteinase inhibitor of the ovalbumin serpin family. *J Mol Biol 293:29.*

26. Wolf, R., A. Mirmohammadsadegh, M. Walz, B. Lysa, U. Tartler, R. Remus, U. Hengge, G. Michel, and T. Ruzicka. 2003. Molecular cloning and characterization of alternatively spliced mRNA isoforms from psoriatic skin encoding a novel member of the S100 family.

27. Mattick, J. S. 2001. Non-coding RNAs: the architects of eukaryotic complexity. *EMBO Rep 2:986.*

28. Mattick, J. S. 2003. Challenging the dogma: the hidden layer of non-protein-coding RNAs in complex organisms. *Bioessays 25:930.*

29. Morey, C., and P. Avner. 2004. Employment opportunities for non-coding RNAs. *FEBS Lett 567:27.*

30. Wienholds, E., W. P. Kloosterman, E. Miska, E. Alvarez-Saavedra, E. Berezikov, E. de Bruijn, R. H. Horvitz, S. Kauppinen, and R. H. A. Plasterk. 2005. MicroRNA Expression in Zebrafish Embryonic Development. *Science:1114519.*

31. Erdmann, V. A., M. Z. Barciszewska, A. Hochberg, N. de Groot, and J. Barciszewski. 2001. Regulatory RNAs. *Cell Mol Life Sci 58:960.*

32. Li, T., J. Spearow, C. M. Rubin, and C. W. Schmid. 1999. Physiological stresses increase mouse short interspersed element (SINE) RNA expression in vivo. *Gene 239:367.*
33. Schmid, C. 1998. Does SINE evolution preclude Alu function? *Nucl. Acids Res. 26:4541.*
34. Eddy, S. R. 2002. Computational genomics of noncoding RNA genes. *Cell 109:137.*
35. Klein, R. J., and S. R. Eddy. 2003. RSEARCH: finding homologs of single structured RNA sequences. *BMC Bioinformatics 4:44.*
36. Liang, P., and A. B. Pardee. 1998. Differential display. A general protocol. *Mol Biotechnol 10:261.*
37. Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. *Molecular Cloning. A Laboratory Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor.
38. Kalman, J., K. Kitajka, M. Pakaski, A. Zvara, A. Juhasz, G. Vincze, Z. Janka, and L. G. Puskas. 2005. Gene expression profile analysis of lymphocytes from Alzheimer's patients. *Psychiatr Genet 15:1.*
39. Puskas, L. G., A. Zvara, L. Hackler, Jr., and P. Van Hummelen. 2002. RNA amplification results in reproducible microarray data with slight ratio bias. *Biotechniques 32:1330.*
40. Pivarcsi, A., M. Szell, L. Kemeny, A. Dobozy, and Z. Bata-Csorgo. 2001. Serum factors regulate the expression of the proliferation-related genes alpha5 integrin and keratin 1, but not keratin 10, in HaCaT keratinocytes. *Arch Dermatol Res 293:206.*
41. Steinberg, T., and R. Burgess. 1992. Tagetitoxin inhibition of RNA polymerase III transcription results from enhanced pausing at discrete sites and is template-dependent. *J. Biol. Chem. 267:20204.*
42. Martignetti, J., and J. Brosius. 1993. BC200 RNA: A Neural RNA Polymerase III Product Encoded by a Monomeric Alu Element. *PNAS 90:11563.*
43. Sesto, A., M. Navarro, F. Burslem, and J. L. Jorcano. 2002. Analysis of the ultraviolet B response in primary human keratinocytes using oligonucleotide microarrays. *PNAS 99:2965.*
44. Schneider, R. J., and I. Mohr. 2003. Translation initiation and viral tricks. *Trends Biochem Sci 28:130.*
45. He, H., F. Dai, L. Yu, X. She, Y. Zhao, J. Jiang, X. Chen, and S. Zhao. 2002. Identification and characterization of nine novel human small GTPases showing variable expressions in liver cancer tissues. *Gene Expr 10:231.*
46. Chen, Y., C. Holcomb, and H. Moore. 1993. Expression and Localization of Two Low Molecular Weight GTP-Binding Proteins, Rab8 and Rab10, by Epitope Tag. *PNAS 90:6508.*
47. Fung, P. A., J. Gaertig, M. A. Gorovsky, and R. L. Hallberg. 1995. Requirement of a small cytoplasmic RNA for the establishment of thermotolerance. *Science 268:1036.*
48. Takeda, K., H. Ichijo, M. Fujii, Y. Mochida, M. Saitoh, H. Nishitoh, T. K. Sampath, and K. Miyazono. 1998. Identification of a Novel Bone Morphogenetic Protein-responsive Gene That May Function as a Noncoding RNA. *J. Biol. Chem. 273:17079.*
49. Brannan, C. I., E. C. Dees, R. S. Ingram, and S. M. Tilghman. 1990. The product of the H19 gene may function as an RNA. *Mol Cell Biol 10:28.*
50. Srikantan, V., Z. Zou, G. Petrovics, L. Xu, M. Augustus, L. Davis, J. R. Livezey, T. Connell, I. A. Sesterhenn, K. Yoshino, G. S. Buzard, F. K. Mostofi, D. G. McLeod, J. W. Moul, and S. Srivastava. 2000. PCGEM1, a prostate-specific gene, is

overexpressed in prostate cancer. *PNAS 97:12216.*

51. Creamer, D., D. Sullivan, R. Bicknell, and J. Barker. 2002. Angiogenesis in psoriasis. *Angiogenesis 5:231.*
52. Detmar, M., L. Brown, K. Claffey, K. Yeo, O. Kocher, R. Jackman, B. Berse, and H. Dvorak. 1994. Overexpression of vascular permeability factor/vascular endothelial growth factor and its receptors in psoriasis. *J. Exp. Med. 180:1141.*
53. Li, T. H., and C. W. Schmid. 2001. Differential stress induction of individual Alu loci: implications for transcription and retrotransposition. *Gene 276:135.*
54. Pivarcsi, A., A. Koreck, L. Bodai, M. Szell, C. Szeg, N. Belso, A. Kenderessy-Szabo, Z. Bata-Csorgo, A. Dobozy, and L. Kemeny. 2004. Differentiation-regulated expression of Toll-like receptors 2 and 4 in HaCaT keratinocytes. *Arch Dermatol Res 296:120.*
55. Morrison, L. A. 2004. The Toll of herpes simplex virus infection. *Trends Microbiol 12:353.*
56. Liu, W. M., W. M. Chu, P. V. Choudary, and C. W. Schmid. 1995. Cell stress and translational inhibitors transiently increase the abundance of mammalian SINE transcripts. *Nucleic Acids Res 23:1758.*
57. Panning, B., and J. R. Smiley. 1995. Activation of expression of multiple subfamilies of human Alu elements by adenovirus type 5 and herpes simplex virus type 1. *J Mol Biol 248:513.*
58. Rudin, C. M., and C. B. Thompson. 2001. Transcriptional activation of short interspersed elements by DNA-damaging agents. *Genes Chromosomes Cancer 30:64.*
59. Hollander, M., I. Alamo, and A. Fornace, Jr. 1996. A novel DNA damage-inducible transcript, gadd7, inhibits cell growth, but lacks a protein product. *Nucl. Acids Res. 24:1589.*
60. Crawford, D. R., G. P. Schools, S. L. Salmon, and K. J. Davies. 1996. Hydrogen peroxide induces the expression of adapt15, a novel RNA associated with polysomes in hamster HA-1 cells. *Arch Biochem Biophys 325:256.*
61. Wang, Y., D. R. Crawford, and K. J. Davies. 1996. adapt33, a novel oxidant-inducible RNA from hamster HA-1 cells. *Arch Biochem Biophys 332:255.*
62. Rogelj, B., and K. P. Giese. 2004. Expression and function of brain specific small RNAs. *Rev Neurosci 15:185.*
63. Allen, T. A., S. Von Kaenel, J. A. Goodrich, and J. F. Kugel. 2004. The SINE-encoded mouse B2 RNA represses mRNA transcription in response to heat shock. *Nat Struct Mol Biol 11:816.*
64. Chen, W., W. Bocker, J. Brosius, and H. Tiedge. 1997. Expression of neural BC200 RNA in human tumours. *J Pathol 183:345.*
65. Iacoangeli, A., Y. Lin, E. J. Morley, I. A. Muslimov, R. Bianchi, J. Reilly, J. Weedon, R. Diallo, W. Bocker, and H. Tiedge. 2004. BC200 RNA in invasive and preinvasive breast cancer. *Carcinogenesis 25:2125.*
66. Ji, P., S. Diederichs, W. Wang, S. Boing, R. Metzger, P. M. Schneider, N. Tidow, B. Brandt, H. Buerger, E. Bulk, M. Thomas, W. E. Berdel, H. Serve, and C. Muller-Tidow. 2003. MALAT-1, a novel noncoding RNA, and thymosin beta4 predict metastasis and survival in early-stage non-small cell lung cancer. *Oncogene 22:8031.*
67. Jacquot, C., D. Carbonnelle, C. Tomasoni, A. Papaconstadinou, V. Roussis, and C. Roussakis. 2004. Identification of a novel putative non-coding RNA involved in proliferation arrest of a non-small cell lung carcinoma cell line treated with an original

chemical substance, methyl-4-methoxy-3-(3-methyl-2-butanoyl) benzoate. *Int J Oncol 25:519.*

68. Petrovics, G., W. Zhang, M. Makarem, J. P. Street, R. Connelly, L. Sun, I. A. Sesterhenn, V. Srikantan, J. W. Moul, and S. Srivastava. 2004. Elevated expression of PCGEM1, a prostate-specific gene with cell growth-promoting function, is associated with high-risk prostate cancer patients. *Oncogene 23:605.*

ACKNOWLEDGEMENTS

First of all I wish to thank Prof. Dr. Attila Dobozy and Prof. Dr. Lajos Kemény for providing me the excellent opportunity to work at the Department of Dermatology and Allergology, University of Szeged.

I thank Dr. Márta Széll for the excellent supervision of the project and for her constant support and Dr. Zsuzsanna Bata for giving me invaluable advice and support.

I am grateful to all the colleagues: Dr. Anna Kenderessy Szabó, Dr. Mária Kiss, Dr. Andor Pivarcsi, Dr. László Bodai, Hilda Polyánka, Csilla Szeg (Department of Dermatology and Allergology, University of Szeged, Szeged), Dr. Klára Megyeri, Prof. Dr. Yvette Mándy (Department of Medical Microbiology and Immunobiology, University of Szeged, Szeged) and Dr. László Puskás (Biological Research Center of the Hungarian Academy of Sciences, Szeged).

I am also very grateful to Dr. József Ongrádi (Department of Dermatology, Semmelweis University, Budapest) for guiding my first steps in scientific research.

I wish to acknowledge the technical help of Andrea Gyimesi and Edit Márta Gordos.