Investigation on molecular mechanisims of epithelial cell stress responses

Summary of PhD thesis

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Szeged

2013

List of abbreviations

CHO-K1	chinese hamster ovarian-K1 cell line
g	glycoprotein
GAG	glycosaminoglycan
GFP	green fluorescence protein
HaCaT	spontaneously immortalized human keratinocyte cell line
HeLa	human epithelial carcinoma cell line
HS	heparan sulfate
HSPG	heparan sulfate proteoglycan
HSV-1	herpes simplex virus type 1
HSV-2	herpes simplex virus type 2
LPS	lipopolysaccharide
ncRNA	non-coding RNA
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NHKs	normal human keratinocytes
ONPG	O-nitrophenyl-b-D-galactopyranoside
PBS	phosphate buffered saline
p.f.u.	relative number of plaques
PRINS	Psoriasis Susceptibility Related RNA Gene Induced by Stress
P/S	penicillin/streptomycin
SD	standard deviation
siRNAs	small interfering RNAs
3-OS HS	3-O-sulfated heparan sulfate
UV	ultraviolet

Publications directly related to the subject of the dissertation

I. **Bacsa S**, Karasneh G, Dosa S, Liu J, Valyi-Nagy T, Shukla D. 2011. Syndecan-1 and syndecan-2 play key roles in herpes simplex virus type 1 infection. J Gen Virol., 92(Pt 4):733-43. **IF: 3.36**

II. Lilla Bari*, **Sarolta Bacsa***, Enikő Sonkoly, Zsuzsanna Bata-Csörgő, Lajos Kemény, Attila Dobozy, Márta Széll. 2011. Comparison of stress-induced PRINS gene expression in normal human keratinocytes and HaCaT cells. Arch Dermatol Res., 303(10):745-52. **IF: 2.27**

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1. Introduction

Stress is widely defined as a harmful factor (biological, chemical or physical), which triggers a series of cellular and systemic events, resulting in reparation of cellular and organismal homeostasis. The cellular stress response can be defined as a reaction to the threat of damage of the structure and function of macromolecules, including proteins, DNA, RNAs and lipids. It includes an evolutionarily highly conserved mechanism that protects cells from environmental stressors such as heat, ultraviolet (UV) light, heavy metals, oxidative and osmotic stress and infection of microbial pathogens. Cells can respond to different types of environmental stress in several ways ranging from activation of cell survival pathways to eliciting programmed cell death. The initial cell response to stressful conditions is the repairing of damaged macromolecules, helping the cell to defend against and recover from the insult serving thus to promote cell survival conditions. If the cellular stress responses fail to repair the damaged macromolecular structure caused by stress, then cell death pathways are activated. The most well-known cell death mechanisms involve apoptosis, pyroptosis, necrosis and in some circumstances autophagic cell death.

Infection with microbial pathogens, such as viruses, bacteria, fungi, and protozoa elicits a diverse array of host protective and stress responses, including cell death and proliferative responses, inflammatory and innate immune responses. During pathogen infection the innate immune system constitutes the first line of host defense and plays a crucial role in the early recognition and subsequent triggering of a pro-inflammatory response. Epithelial cells act as front-line defense executioners against microbial invasion by using pathogen recognition receptors (PRRs) including TLRs, NOD-like receptors (NLRs), retinoic acid-inducible gene (RIG-I) - like receptors (RLRs), retinoic acid inducible gene-I like RNA helicases (RLHs) and C-type lectin receptor to recognize pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs). Following ligand recognition, PRRs present signal to the host and trigger proinflammatory and antimicrobial response by activating several intracellular signaling pathways, including kinases and transcription factors resulting the activation of gene expression and synthesis of a broad range of molecules, including cytokines, chemokines, cell adhesion molecules, and immunoreceptors. All of these mechanisms build up the early host response to infection and at the same time represent an important link to the adaptive immune response.

The goal of our work was to investigate the epithelial cell stress responses following viral and bacterial infection using two model systems.

First, we examined the role of two heparan sulfate proteoglycans, (HSPGs) syndecan-1 and syndecan-2 in herpes simplex virus-1 (HSV-1) infection. Since syndecan-1 and syndecan-2 are relatively common HSPGs found on the target epithelial cells for HSV-1 infection and several microbial pathogens can alter syndecan-1 expression we aimed to explore the role of syncecan-1 and syndecan-2 core proteins in HSV-1 infection. Using protein expression studies we tested whether HSV-1 infection has an effect on syndecan-1 and syndecan-2 protein expression level in human cervical cell line (HeLa). Since heparan sulfate (HS) plays an important role in HSV-1 entry as an attachment receptor, the role of specific proteoglycan core proteins in the infection process remains poorly understood. Therefore we performed *in vitro* studies using HeLa cells to determine the effect of syndecan-1 and syndecan-2 gene silencing on viral entry, plaque formation and cell survival using small interfering RNAs (siRNAs) specific for HSPGs.

Second, our aim was to explore the role of Psoriasis Susceptibility Related RNA gene Induced by Stress (PRINS) in bacterial LPS induced cellular stress response in both immortalized HaCaT cells and in normal human keratinocytes (NHKs). We recently established that HaCaT cells and NHKs exhibit different PRINS expression patterns upon translation inhibition, UV-B and LPS induction and upon treatment with several microbial pathogens using quantitative real time RT-PCR methods. To identify whether this differential PRINS expression caused by the alteration of nuclear factor-κB (NF-κB) signal transduction pathway and whether PRINS was involved as an upstream regulator of it we silenced the PRINS gene expression with siRNAs in both HaCaT cells and in NHKs and monitored NF-κB signal transduction following LPS treatment.

2. Aims

2.1 To investigate the role of syndecan-1 and syndecan-2 in herpes simplex virus type-1 infection

- To examine the impact of HSV-1 infection on syndecan-1 and syndecan-2 protein synthesis and heparan sulfate expression on the human epithelial surface

- To investigate the effect of syndecan-1 and syndecan-2 gene silencing by RNA interference on HSV-1 entry, plaque formation and cell survival in human epithelial cells

2.2 To monitor whether PRINS is involved as an upstream regulator in the regulation of the NF-κB signal transduction pathway in normal human keratinocytes and in the immortalized keratinocyte cell line, HaCaT

- To explore whether the silencing of PRINS expression has an effect on the LPS-induced NF-κB response either in HaCaT cells or in NHKs

3. Material and methods

3.1 Transfection methods

HeLa and CHO-K1 cells were transfected at 80% confluence at a concentration of 200 nM per well with syndecan-1 siRNA (59-CCAUUCUGACUCGGUUUCU[dT][dT]-39, and 59-GCCAAGGUUUUAUAAGGCU[dT][dT]-39; syndecan-2 siRNA (SASI_Hs0100195372, SASI_Hs0100195365; or a non-specific, scrambled control siRNA (59-GAUCAUACGUGC GAUCAGA[dT][dT]-39.

The plasmids used in the detection of LPS-induced NF-κB activity experiment were pNF-κB-Luc, pGL4.75[hRluc/CMV], pSilencer 2.1-U6 hygro and pmaxGFP. To knock down PRINS expression, transient transfection experiments using a vector-based RNA interference method were carried out both with the HaCaT cell line stably transformed with the NF-κB-luciferase chimeric construct and with NHKs. NF-κB-HaCaT cells were transfected with the PRINS silencing pSilencer 2.1-U6 construct (AK696) and a construct harboring a scrambled sequence of PRINS gene (SC1313), was used as a control. NHKs were simultaneously co-transfected with the NF-κB-luc plasmid and either with the PRINS silencing pSilencer 2.1-U6 construct (AK696) or with control construct (SC1313).

3.2 Viral entry assay and antibody blocking assay

HeLa cells and HSV-1 entry receptor defficient CHO-K1 cells were transfected with syndecan-1, syndecan-2 or scrambled siRNA. After 48 h, cells were infected with the β-galactosidase expressing recombinant HSV-1(KOS) gL86. β-galactosidase expression is driven by HSV-1 ICP4 promoter by early viral protein synthesis upon HSV infection. At 6 h post-infection, the soluble substrate O-nitrophenyl-b-D-galactopyranoside was added and enzymic activity was measured.

Confluent HeLa cells were incubated with serial dilutions of rabbit pAbs to sydecan-1, syndecan-2 or control anti-myc mAb. HSV-1(KOS) gL86 (m.o.i. of 10) was then added to the cells. After 2 h, the bound viruses were removed and then incubation was continued for another 3 h. β-galactosidase activity was measured by adding its substrate ONPG.

3.3 Plaque assay and cytotoxicity assay

HeLa cells were transfected with syndecan-1, syndecan-2 or scrambled siRNA. At 48 h post-transfection, cells were infected (m.o.i. of 0.01) with HSV-1 (KOS) or mock infected in PBS. Cells were then washed with PBS and fresh medium was added. Cells were incubated for 72 h at 37 ºC. After 72 h, cells were fixed with 100% methanol and stained with the Giemsa stain. Infectivity was assessed by counting plaques formed using a 10x objective lens of an inverted light microscope.

HeLa cells were transfected with syndecan-1, syndecan-2 or scrambled siRNA. In addition to the non-specific, scrambled siRNA transfected cells, non-transfected cells were also used as controls. 48 hours post-transfection, cells were infected with HSV-1(KOS) (m.o.i. of 0.01). The inoculum was then removed by washing the cells with PBS and fresh medium was added. Cells were incubated at 37 ºC for 120 h, then fixed with 100% methanol and stained with Giemsa stain. The number of dead cells was determined using NIH Image J software at twenty high power fields (x 40 objective).

3.4 Flow cytometry

For syndecan-1, syndecan-2 and HS cell surface expression, confluent monolayers of HeLa cells were infected with HSV-1 (KOS). Cells were then washed with PBS, harvested and incubated with the respective primary antibody (mouse anti-syndecan-1 mAb at 1 µg per $1x10^6$ cells, rat anti-syndecan-2 mAb at 1.25 µg per $1x10^6$ cells, or mouse anti-human HS mAb 10E4 epitope). After primary antibody incubation, cells were washed and incubated with goat anti-mouse, goat anti-rat-FITC-conjugated secondary anti-IgGs or goat anti-mouse-FITC-conjugated IgM. Cells stained only with goat anti-mouse-FITC or goat anti-rat-FITC or goat anti-mouse- FITC-conjugated IgM were used as background controls.

3.5 Immunoblotting

Approximately 150–200 µg of total cell protein in lysis buffer was incubated with 100% methanol overnight and with 100% acetone and the protein pellet was redissolved in

heparinase buffer (0.1 M NaOAc+0.1 mM CaOAc, pH 7.0). GAGs were digested with heparinase I, II, III (12 µg per sample) and chondroitinase ABC (0.005 U per sample). Samples were then denatured in Laemmli Sample Buffer with β-mercaptoethanol and heated to 96 ºC for 10 min before loading onto a SDS-PAGE gel. Separated proteins were then transferred to nitrocellulose membrane, blocked and incubated with primary rabbit pAbs against sydecan-1 and syndecan-2. The blots were rinsed and incubated with HRP-conjugated goat anti-rabbit IgG. Anti-β-actin mouse mAb as the primary antibody and HRP-conjugated rabbit anti-mouse IgG as the secondary antibody were used for detecting β-actin as a loading control. The signal was visualized with SuperSignal West Femto maximum sensitivity substrate and the blots were exposed to X-ray film. Developed films were scanned and protein bands were quantified using the NIH Image J software.

3.6 Detection of LPS-induced NF-κB activity

Luciferase assays were performed to determine the NF-κB activity in response to LPS stimulation of the NF-κB-HaCaT cells and NHKs. To assay the effect of LPS induction on NF-κB promoter activity, NF-κB-HaCaT cells were transfected with the PRINS silencing construct and NHKs were co-transfected with NF-κB-luc plasmid, pGL4.75 [hRluc/CMV] plasmid and pSilencer 2.1-U6 plasmid construct. After transfection, cells were incubated with 2.5 µg/ml LPS then cells were lysed and the luciferase activities were measured with the Promega Luciferase Assay System. Firefly and Renilla luciferase activities were measured in a Thermo Luminoskan Ascent Machine.

3.7 Real-time reverse transcriptase PCR

For real time reverse transcriptase PCR (RT-PCR), 1 µg of purified total RNA was reverse transcribed by using the iScript kit. After reverse transcription, real time 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.

3.8 Statistical analyses

Statistical analyses were performed with the STATISTICA software (version 8.0) for windows. Normality was tested using the Kolmogorov–Smirnov test. All variables were distributed normally. Homogeneity of variance was determined using Levene's test and was considered violated when this test yielded $p<0.05$. All variances were homogeneous. Data were assessed using ANOVA followed by Scheffe's post-hoc test to evaluate the effects of gene silencing of syndecan-1 and syndecan-2 on HSV-1 viral entry, plaque formation and cytotoxicity. To evaluate the effects of silencing of PRINS on LPS-induced NF-κB activity data were assessed using the repeated measures ANOVA.

4. Results

4.1 HSV-1 infection in HeLa cells enhances syndecan-1 and syndecan-2 cell surface expression

To determine whether HSV-1 infection affects cell surface expression of syndecan-1 and syndecan-2, the expression level of syndecan-1 and syndecan-2 on HeLa cell surface was analyzed at various times after HSV-1(KOS) infection by flow cytometry. Cell surface expression levels of syndecan-1 and syndecan-2 in mock-infected HeLa cells were used as controls. Both syndecan-1 and syndecan-2 cell surface expressions were significantly upregulated as soon as 2 h after HSV-1 infection. The increases were also observed at 4 and 6 h post-infection in HeLa cells.

4.2 HSV-1 infection in HeLa cells enhances syndecan-1 and syndecan-2 protein synthesis

Since HSV-1 infection enhances syndecan-1 and syndecan-2 cell surface expression, this enhancement could be a result of protein synthesis induction or a higher redistribution of HSPGs on the cell surface. To determine if HSV-1 infection modulates syndecan-1 and syndecan-2 expression at the protein level, Western blot analysis was performed on HeLa cells. Mock-infected cells were used as controls. Densitometric analysis showed that the expression level of syndecan-1 protein was increased by 1.64 ± 0.16-fold at 2 h and 2.01 ±

0.16-fold at 6 h after HSV-1 infection. Syndecan-2 protein expression level was also increased by 2.59 ± 0.64-fold at 2 h and 3.16 ± 1.02-fold at 6 h after HSV-1.These results demonstrate that HSV-1 not only enhances the cell surface distribution of syndecans, but also induces *de novo* syndecan-1 and syndecan-2 protein synthesis.

4.3 HSV-1 infection causes an increase of HS expression on cell surface

Since HSV-1 infection of host cells results in an upregulation of protein expression and cell surface deposition of syndecans, we aimed to determine whether this may lead to an upregulation of HS as well. To investigate if HSV-1 infection affects HS cell surface expression, flow cytometry analysis was performed. At 2 h post-infection, HS surface expression increased in cells treated with HSV-1(KOS) compared to those that were mock treated. However, at later time points, the increase of HS surface expression exhibited a plateau and did not show the dynamic increase we observed for syndecan-1 and syndecan-2 upregulation after HSV-1 treatment.

4.4 Syndecan-1 and syndecan-2 downregulation in HeLa cells

We have demonstrated the significance of syndecan-1 and syndecan-2 on HSV-1 infection with selective gene silencing of syndecan-1 and syndecan-2 in HeLa cells using siRNA expression constructs. Gene silencing of syndecan-1 and syndecan-2 was detected at the protein level by using Western blot analysis. Densitometric analysis showed that treatment with syndecan-1- and syndecan-2-specific siRNA resulted in a significant reduction in syndecan-1 and syndecan-2 protein expression, respectively.

4.5 Downregulation of syndecan-1 and syndecan-2 inhibits HSV-1 entry

The effect of reduced syndecan-1 and syndecan-2 protein levels on HSV-1 entry into HeLa cells was examined. Viral entry was compared in cells treated with syndecan-1 or syndecan-2 siRNA with those treated with scrambled siRNA or mock treated. A statistically significant, 26.6 ± 4.6% inhibition of HSV-1 entry was observed in cells transfected with

syndecan-1 siRNA ($p<0.05$). Transfected cells with syndecan-2 siRNA resulted in an even more significant, 64.9 ± 5.5% inhibition of HSV-1 entry ($p<0.0001$).

4.6 Anti-syndecan-1 and anti-syndecan-2 polyclonal antibodies block HSV-1 entry

pAbs specific for the extracellular region of syndecan-1 or syndecan-2 were able to block HSV-1 entry into HeLa cells in a dose-dependent manner. At the maximum pAb concentration (4 µg per well) the observed inhibitions were 35.7 ± 4.2 % and 57.84 ± 6.5 %, respectively, compared with HeLa cells that were treated with a control, anti-myc mAb.

4.7 Downregulation of syndecan-1 and syndecan-2 inhibits plaque formation, reduces the size of HSV-1 plaques and enhances cell survival

The ability of HSV-1 to form plaques reflects its ability to enter cells, replicate and spread to infect neighbouring uninfected cells. There was a clear and significant reduction in the number of plaques formed in HeLa cells transfected with syndecan-1 (63.22 ± 2.65%) and syndecan-2 (98.73 ± 5.78%) specific siRNAs compared with mock treated or scrambled siRNA-transfected cells. To prove that the reduction in entry and plaque formation is an effect of syndecan downregulation, and not a result of increased cell death, dead cells in each condition were counted 120 h after HSV-1 infection and the numbers were normalized to those observed with mock-treated cells. Downregulation of syndecan-1 reduced the percentage of dead cells after HSV-1 infection (by 33.06 ± 5.89 %), and downregulation of syndecan-2 resulted in a statistically significant decline in the percentage of dead cells (by 80.45 ± 5.68 %).

4.8 Silencing of PRINS expression does not affect the LPS-induced NF-κB response either in HaCaT cells or in NHKs

HaCaT cells and NHKs exhibited different PRINS expression patterns subsequent to various forms of stress induction: while translation inhibition, UV-B irradiation and co-incubation with microbial compounds. We set out to investigate whether the differences in

PRINS-NF-κB interactions might be responsible for the differential PRINS expression in these two cell types. Our results indicated that gene-specific PRINS silencing did not affect the LPS-induced NF κB activity either in HaCaT cells or in NHKs. LPS treatment induced a significant three- to four-fold NF-κB activation in HaCaT cells and in NHKs, but this induction was not affected by PRINS silencing in either cell types.

5. Discussion

Investigations on the molecular mechanisms of epithelial cell stress responses to pathogen invasion has great importance for our better understanding of the pathogenesis of diseases and indentifying novel therapeutic targets. Our aim was to investigate two aspects of stress responses of epithelial cells triggered by viral or bacterial induction.

In our first model system, HSV-1 was chosen as a viral induction agent since it is a clinically important pathogen and leads to numerous diseases from oral lesions to more severe conditions. Currently, no cure exists against HSV-1 therefore expansion of our understanding of HSV-1–host cell interactions has a great significance on the prevention and treatment of HSV-1 viral infection. The initial contact of HSV-1 with its principal target epithelial cells is the binding of the virus to HS chains which are expressed on the cell surface as HSPGs. HS can serve as a receptor for a wide range of microbial pathogens, including viruses and bacteria Although the role of HS is well studied as an attachment receptor for HSV-1, the role of HSPG core proteins in HSV-1 infection is poorly understood. Since predominant HSPGs on human epithelial cell surfaces are syndecan-1 and syndecan-2, we aimed to explore the role of their core proteins in HSV-1 infection. The first part of our studies directly implicates two members of the syndecan family of HSPGs, syndecan-1 and syndecan-2, as important mediators of HSV-1 infection.

We demonstrated that HSV-1 infection in HeLa cells enhances both syndecan-1 and syndecan-2 cell surface expression, occurs both at the cell surface level and also at the protein synthesis level. Interestingly, our findings suggest that an increase in syndecan-1 and syndecan-2 expression levels, although important for many reasons, may also be used as a marker for active HSV-1 infection. Further experiments are needed to understand mechanisms

by which HSV-1 upregulates syndecan-1 and syndecan-2 expression and to determine whether this upregulation is a result of specific signaling pathway activation by the virus.

Additionally, we demonstrate that both syndecan-1 and syndecan-2 contribute significantly to viral entry and spread. Knocking down of either syndecan-1 or syndecan-2 shows detectable effects on HSV-1 entry and plaque formation. Our results also suggest that syndecan-2 may have a distinctly larger role in HSV-1 infection than syndecan-1. In addition, we provide evidence to directly implicate the HSPG core protein in viral entry. Many known protein receptors for HSV-1 entry can be blocked by antibodies, which in turn, blocks viral entry. Similar to those receptors, we also found that pAbs against synedecan-1 and syndecan-2 block entry. While it is quite possible that antibodies may act by producing steric hinderance to virus binding *via* HS, it is also possible that the core protein may directly interact with HSV-1 glycoproteins and that interaction is blocked by the antibodies. Alternatively, a second possibility is that the pAbs may be able to block low affinity interactions (or create steric hindrance) that do not involve conformational changes. Our results, nevertheless, highlight that syndecan-1 and syndecan-2 both play a critical role during HSV-1 entry and that the two HSPGs show detectable differences in their abilities to facilitate infection. A related interesting finding was that the effect of syndecan-2 knockdown was even more severe at the plaque formation level. Unlike entry, the downregulation of syndecan-2 expression almost completely inhibited plaque formation in HeLa cells. A reduction in plaque number was expected since we found that downregulation of syndecan-1 and syndecan-2 reduces HSV-1 entry. However, the observed dramatic reduction in plaque formation raises the possibility that reduced virus entry may not be the only reason for reduced plaque formation and that an additional role for syndecan-2 in HSV-1 replication or spread could not be ruled out. Since syndecans participate in endocytosis, they may affect virus transport as well. One possible way to explain the fact that syndecan-2 has a more significant role in HSV-1 entry is related to the differences in GAG distribution on the ectodomains of the HSPGs. While the syndecan-1 ectodomain carries HS and chondroitin sulfate (CS) chains, syndecan-2 carries solely HS chains on its ectodomain. It is possible that the presence of HS alone may help reduce any non-specific virus binding generated by similarly charged but less effective CS. Further understanding of the role of the syndecans in HSV-1 infection could identify novel antiviral targets and lead to the development of improved antiviral strategies.

In our second model system, we aimed to elucidate the putative role of a novel non-coding RNA, PRINS in NF-κB signaling pathway activation in human keratinocytes following bacterial LPS induction as an epidermal stress factor.

Previously, we identified a novel non-coding RNA (ncRNA), PRINS that was overexpressed in the non-involved epidermis of psoriatic patients as compared with epidermis of healthy individuals. Various stress signals, such as serum starvation, contact inhibition, UV-B irradiation, viral (HSV-1) infection and translational inhibition by cycloheximide, induced the expression of PRINS in HaCaT cells. The effects of stressors were apparent as early as 0.5–3 h after the application of stress, indicating that PRINS may play a role in the early phase of the cellular stress response.

In a next set of experiments we compared PRINS expression patterns in HaCaT cells and NHKs. We analyzed PRINS expression after treatment with cycloheximide (a chemical inhibitor of mRNA translation), UV-B irradiation and incubation with several microbial compounds inluding *Candida albicans*, LPS, *Staphylococcus aureus* peptidoglycan or *Mycobacterium tuberculosis* extract in both NHKs and HaCaT cells. We found that PRINS expression responded differentially to various stress signals and microbial agents in HaCaT cells and in NHKs. Incubation of HaCaT cells and NHKs with cycloheximide resulted in elevations of PRINS expression of similar magnitude, but with different time courses in the two cell types. Exposition of HaCaT cells and NHKs to UV-B irradiation resulted in a gentle, prolonged PRINS expression response in HaCaT cells and a late, significant PRINS expression response in NHKs, respectively. The most striking differences we saw between the two cell types in PRINS expression were after incubation with microbial compounds: while PRINS expression in HaCaT cells was highly elevated in response to all of applied microbial agents, a significant elevation of PRINS expression in NHKs was observed only after incubation with LPS. We supposed that detected differences in microbial compound-induced PRINS expression may not be related to TLR-mediated signal transduction events in these two cell types. Since NF-κB is considered to be a down-stream effector in signal transduction, we investigated whether PRINS is involved in the regulation of signaling events leading to NF-κB activation. To reveal whether NF-κB transduction pathway is affected by the PRINS non-coding RNA, we silenced the PRINS gene expression with siRNA in NHKs and HaCaT keratinocytes and monitored NF-κB signal transduction after LPS treatment. Our results

demonstrated that PRINS gene silencing had no effect on NF-κB activation either in NHKs or in HaCaT cells, suggesting that PRINS is not an upstream effector of NF-κB signalling in keratinocytes. To identify which signal transduction events are affected by the PRINS ncRNA, further studies are needed.

Human epithelial surfaces have a great importance in defense mechanisms against invading organisms. Infection of epithelial cells by microbial pathogens activates numerous host protective molecular mechanisms which are coordinated by a complex program of gene expression. A better understanding of HSV-1/host interaction mechanisms and the role of a novel ncRNA in the pathogenesis of psoriasis will be useful for screening and evaluating possible therapeutic strategies and may provide some novel base for development of new strategies for the treatment of infectious and inflammatory diseases.

Acknowledgements

I would like to thank Marta Szell, DSc and Deepak Shukla, PhD for their support and professional supervision of my scientific work.

I wish to thank the Rosztoczy Foundation and Professor Lajos Kemény for providing me the excellent opportunity to work at the Department of Ophthalmology and Visual Sciences at University of Illinois at Chicago and at the Department of Dermatology and Allergology at University of Szeged.

I am very grateful to Tibor Valyi-Nagy, MD, PhD continues support and encouragement.

Thank to Myung-Jin Oh, Ghadah Karasneh, Dr. Bernadett Kormos, Dr. Attila Bebes, Gábor Tax, Dr. Gergő Groma, Dr. Kornélia Szabó, Andrea Tanácsné Bajkán and Éva Dósa Rácz and to all colleagues who provided me a lot of help in my work.

My special thanks to my husband for his support and continuous encouragement.

Publication of Bari et al. is supported by the European Union and co-funded by the European Social Fund through the TÁMOP-4.2.2.A-11/1/KONV-2012-0035 European Union Grant.