INVESTIGATIONS ON MOLECULAR MECHANISMS OF EPITHELIAL CELL STRESS RESPONSES

Sarolta Bacsa

Ph. D. Thesis

Szeged

2013

Supervisor:
Márta Széll, DSc

Department of Dermatology and Allergology, Faculty of Medicine, University of Szeged
Dermatological Research Group of the Hungarian Academy of Sciences
Department of Ophthalmology and Visual Sciences, University of Illinois at Chicago

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**
* These two authors contributed equally to this work

Other publications

I. Kovacs SK, Tiwari V, Prandovszky E, Dosa S, **Bacsa S**, Valyi-Nagy K, Shukla D, Valyi-Nagy T. 2009. Expression of herpesvirus entry mediator (HVEM) in the cornea and trigeminal ganglia of normal and HSV-1 infected mice. Current Eye Research, 34:896-904 **IF: 1.51**

II. Valyi-Nagy K, Dosa S, Kovacs SK, **Bacsa S**, Voros A, Shukla D, Folberg R, Valyi-Nagy T. 2010. Identification of virus resistant tumor cell subpopulations in three dimensional uveal melanoma cultures. Cancer Gene Ther., 17(4):223-34.
IF: 3.74

III. Kormos B, Belső N, Bebes A, Szabad G, **Bacsa S**, Széll M, Kemény L, Bata-Csörgő Z. 2011. In Vitro Dedifferentiation of Melanocytes from Adult Epidermis. PLoS One, 23; 6(2):e17197. **IF: 4.09**

IV. Dosa S, Castellanos K, **Bacsa S**, Gagyi E, Kovacs SK, Valyi-Nagy K, Shukla D, Dermody TS, Valyi-Nagy T. 2011. Chronic progressive deficits in neuron size, density, and number in the trigeminal ganglia of mice latently infected with herpes simplex virus. Brain Pathol., 21(5):583-93. **IF: 3.99**

V. Szegedi K, Göblös A, **Bacsa S**, Antal M, Németh IB, Bata-Csörgő Z, Kemény L, Dobozy A, Széll M. 2012 Expression and Functional Studies on the Noncoding RNA, PRINS. Int J Mol Sci., 14(1):205-25. **IF: 2.46**

TABLE OF CONTENTS

List of abbreviations

ANOVA	analysis of variance
BSA	bovine serum albumin
CHO-K1	chinese hamster ovarian-K1 cell line
CS	chondroitin sulfate
DMEM	Dulbecco's modified Eagle's medium
g	glycoprotein
GAG	glycosaminoglycan
GFP	green fluorescence protein
GWAS	Genome-wide association scans
HaCaT	spontaneously immortalized human keratinocyte cell line
HeLa	human epithelial carcinoma cell line
HS	heparan sulfate
Hsp	heat shock protein
HSPG	heparan sulfate proteoglycan
HSV-1	herpes simplex virus type 1
HSV-2	herpes simplex virus type 2
IκB	inhibitor κB
LPS	lipopolysaccharide
mAbs	monoclonal antibodies
mRNA	messenger RNA
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
pAbs	polyclonal antibodies
PBS	phosphate buffered saline
p.f.u.	relative number of plaques
pmaxGFP	plasmid encoding the enhanced green fluorescent protein eGFP
PRINS	Psoriasis Susceptibility Related RNA Gene Induced by Stress
PRRs	pathogen recognition receptors

p53	tumor protein 53
PSORS	psoriasis susceptibility locus 1
P/S	penicillin/streptomycin
SD	standard deviation
siRNAs	small interfering RNAs
3-OS HS	3-O-sulfated heparan sulfate
TGF-α	transforming growth factor-α
TLRs	toll-like receptors
UV	ultraviolet
Vero	African green monkey kidney cell line
VPs	viral proteins

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. Organisms have developed a broad range of stress response mechanisms to cope with stress conditions, acting at the cellular or organelle-specific level.

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. However, if the damage is too severe to be repaired, then cells activate stress signaling cascades to mediate cell death pathways [1]. Although the reactions of cells to environmental stress factors depend on the type and duration of the insult, cells respond rapidly and transiently to all types of stress by activation of four basic mechanisms, all of which are aimed at stabilizing macromolecular structures and functions [2]. The transient cellular stress responses are mediated through:

(1) induction of cell cycle checkpoint control mechanisms leading to cell growth arrest – cell cycle checkpoints are triggered by genotoxic stress in eukaryotic cells such as the G1/S checkpoint [3], the G2/M checkpoint [4] and translational control mechanisms [5];

(2) induction of molecular chaperons, including the heat-shock proteins (Hsps), – Hsps are commonly activated either by induction [6] or by post-translational modification such as phosphorylation, i. e. Hsp27 is known to be activated by phosphorylation *via* the p38 MAP kinase signaling pathway [7];

(3) activation of mechanisms for nucleic acid stabilization and repair – for instance tumor protein 53 (p53) pathway [8] and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway [9] are activated as part of the eukaryotic DNA damage response;

(4) clearance of damaged macromolecules generated by stress – in response to cellular stress signals including heat shock, oxidative damage or microbial infection, heat shock proteins, such as Hsp27 and Hsp90 have a significant role in increasing the activity of the ubiquitin/proteasome system to mediate the degradation of damaged proteins [10].

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. Apoptosis, one type of noninflammatory programmed cell death is triggered through two different pathways, the intrinsic (mitochondria-mediated) pathway and the extrinsic (receptor-mediated) pathway [11]. Apoptosis is regulated by many proteins, such as caspases (cysteine-dependent aspartate-specific protease), B-cell lymphoma 2 (Bcl-2) -related proteins, and p53 [12]. Although several proteins contribute to the control of apoptosis, caspases are believed to be the most significant participants of this process. Activity of caspases lead to many of the morphologic features of apoptotic cell death, including membrane blebbing, polynucleosomal DNA fragmentation, nuclear shrinking and to the loss of overall cell shape [13, 14]. When apoptosis is inhibited, an alternative programmed cell death mechanism, the autophagic cell death is activated [15]. Autophagy (self-eating) is a multistep process characterized by the vesicular sequestration and degradation of unnecessary or dysfunctional cellular components through the lysosomal machinery [16]. Although the interplay between apoptosis and autophagy is poorly understood, several studies suggest that autophagy and apoptosis are antagonistic events that tend to inhibit each other [17, 18]. Necrosis, the additional type of cell death mechanisms has long been considered as an accidental and uncontrolled mode of cell death. Recently it has become clear that the execution of necrotic cell death is also molecularly regulated by a set of signaling pathways. These pathways are also associated with pathological conditions such as ischemia-reperfusion (IR) injury, neurodegeneration and pathogen infection [19-23]. For instance, tumor necrosis factor receptor 1 **(**TNFR1), and toll-like receptors (TLRs) have been reported to trigger necrosis [19] and the serine/threonine kinase receptor-interacting protein 1 and 3 (RIP1 and RIP3) have been described as key mediators of the initiation of necrosis induced by ligand-receptor interactions [11, 24]. Necrosis is characterized by membrane rupture, nuclear swelling, and the release of cellular contents and is accompanied by caspase-independent

inflammation. Finally, pyroptosis is a form of inflammatory programmed cell death associated with antimicrobial responses during inflammation. In contrast to apoptosis, pyroptotic cell death is coordinated by inflammasome-mediated caspase-1 activation and accompanied by membrane rupture, DNA fragmentation and the release of pro-inflammatory cytokines, including interleukin-1β and interleukin-18 (IL-1β and IL-18) [25].

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. To sacrifice pathogen infected cells for the benefit of the remaining tissue is one of the main immune defense mechanisms of multicellular organisms [25]. For instance, in response to bacterial infection, programmed cell death, such as apoptosis, necrosis and pyroptosis are induced as a host innate immune response (Fig.1) [11]. 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 [26]. Human body surfaces are defended by epithelia, which provide a physical barrier between the internal milieu and the external world that contains pathogens. 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) [27-29]. PAMPs are microbe-specific molecules including bacterial carbohydrates, i. e. lipopolysaccharide (LPS) and mannose, bacterial peptides, like flagellin, nucleic acids including bacterial DNA or viral RNA or DNA, bacterial peptidoglycans, lipoteichoic acids, lipopeptides, viral glycoproteins, like hemagglutinin and glycoprotein-41 and fungal glucans which elicit immune response [30]. 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 [30]. 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.

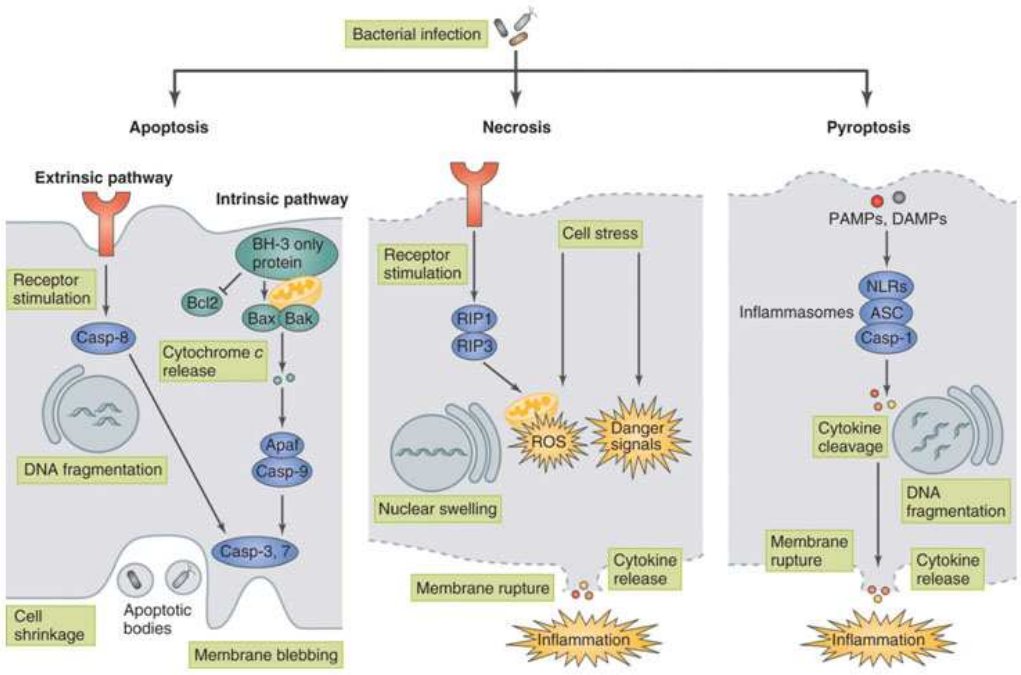


Figure 1. **Bacteria-induced host cell death.** Bacteria induce host cell death through several distinct modalities, including apoptosis, necrosis, and pyroptosis [11].

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 [31, 32] and previous studies have shown that several microbial pathogens can alter syndecan-1 expression [33-35] 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 studies have shown that heparan sulfate (HS) plays an important role in HSV-1 entry as an attachment receptor [36], 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 [37, 38]. 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.

1.1 Herpes simplex virus type-1 and host cells interaction

1.1.1 Herpes simplex viruses

The *Herpesviridae* family forms a large and diverse group consisting over 100 double-stranded DNA viruses of three subfamilies designated as *Alphaherpesvirinae*, *Betaherpesvirinae*, and *Gammaherpesvirinae* [36]. Herpes simplex viruses (HSV): type-1 (HSV-1) and type-2 (HSV-2) are part of the *Alphaherpesvirinae* subfamily of *Herpesviridae* family. The herpes simplex virion consists of a linear, double-stranded DNA genome (152 kb), packaged into an icosahedral capsid that consists of 162 capsomeres with six different viral proteins (VPs) present on the surface. The icosahedral capsid is surrounded by a layer of 22 proteins called the tegument and a lipid bilayer envelope derived from the host cell membrane in which most cell proteins have been replaced by viral membrane proteins. The envelope is composed of 16 viral proteins, including 12 different glycoproteins (gB, gC, gD, gE, gG, gH, gI, gJ, gK, gL, gM, and gN) [39, 40] (Fig. 2). The protected DNA genome and

the coordinated action of at least four glycoproteins: D (gD), B (gB) and the heterodimer H (gH) and L (gL) were shown to be essential for viral infectivity [39].

HSV occur worldwide and have no seasonal variation. Although HSV is normally isolated only from humans, many animal species can be experimentally infected, and many types and species of cultured cells support HSV replication [36, 41]. HSVs are neurotropic viruses producing a lifelong infection by establishing latency in the host sensory neurons of the peripheral nervous system and replicating in epithelial cells during primary infection and reactivation [42]. HSV cause a number of diseases ranging from oral and genital lesions to more severe conditions, such as herpetic stromal keratitis (HSK) a chronic inflammation of the cornea, the main non-traumatic cause of corneal blindness; meningitis and encephalitis [43-45]. The most common forms of disease caused by HSV are manifested as mucocutaneous lesions, which usually occur near the mouth (cold sores or fever blisters), on the cornea (keratitis), or on genital tissues. Oral and ocular lesions are primarily caused by HSV-1, while most genital HSV infections are caused by HSV-2; however, both strains have the ability to cause infection in either area of the body [36, 41]. Genital HSV-1 infections are usually less severe and less prone to recur than those caused by HSV-2 [41]. Less frequently, HSV can also cause life-threatening disease affecting vital organs, including encephalitis in apparently healthy adults, meningitis, myelitis, radiculitis and disseminated disease in infants. The viruses isolated from adult encephalitis cases, keratitis and facial lesions are usually HSV-1, whereas those isolated from cases of neonatal disease, adult meningitis and genital lesions are usually HSV-2 [36, 41]. Many demographic factors affect acquisition of HSV infection. In less developed countries, HSV-1 seroconversion happens early in life – at the age of 5 years in around one third of children, and in around 70–80% by adolescence. In comparison, middle-class and upper-class individuals in more developed countries become infected later on – seroconversion occurs in about 20% of children younger than 5 years; then no substantial rise in incidence happens until an increase to 40–60% at the age between 20 to 40 years [41]. HSV-2 seroprevalence rises from about 20–30% at the age between 15 to 29 years to 35–60% by age 60 years [46]. Since the virus that causes the primary lesions establishes latent infections in sensory or autonomic peripheral neurons and can be reactivated by appropriate stimuli, periodic recurrences of herpetic lesions are common and present one of the troublesome aspects of infections with HSV [36]. A wide variety of environmental

events can trigger the reactivation of the virus, including emotional or physical stress, hormonal changes, menstruation, fever, exposure to sunlight or ultraviolet (UV) light and tissue damage [41].

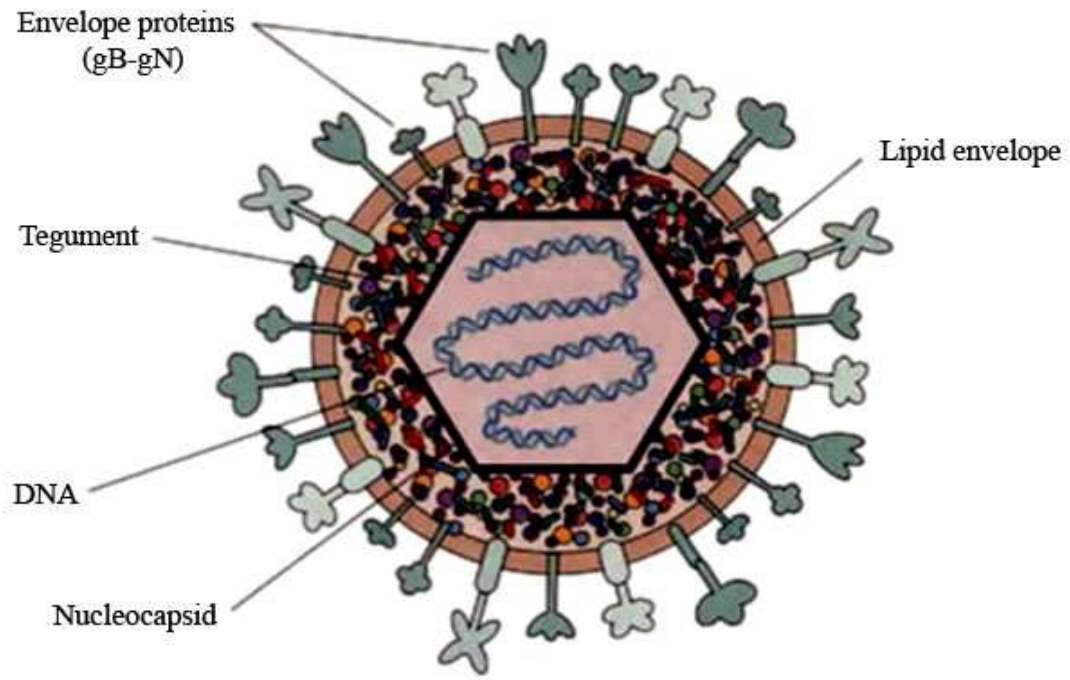


Figure 2. Schematic representation of HSV structure [http://www.manufacturingchemist.com].

1.1.2 The entry of HSV-1 into host cells

HSV-1 entry into host cells marks the first and possibly most critical step in viral pathogenesis that is a result of fusion between the viral envelope and the membrane of the host cell. This process is mediated by the action of five viral glycoproteins (gB, gC, gD, gH and gL) along with their interactions with their cognate receptors [47] (Fig. 3). However, only four of these glycoproteins, such as gB, gD, gH, and gL are necessary and sufficient to allow viral fusion with the host cell plasma membrane [48-50]. Although gC is dispensable for the

infection of cultured cells; either gC or gB can mediate the binding of HSV-1 to cells; gC presence can increase the efficiency of virus binding almost 10-fold [49, 51].

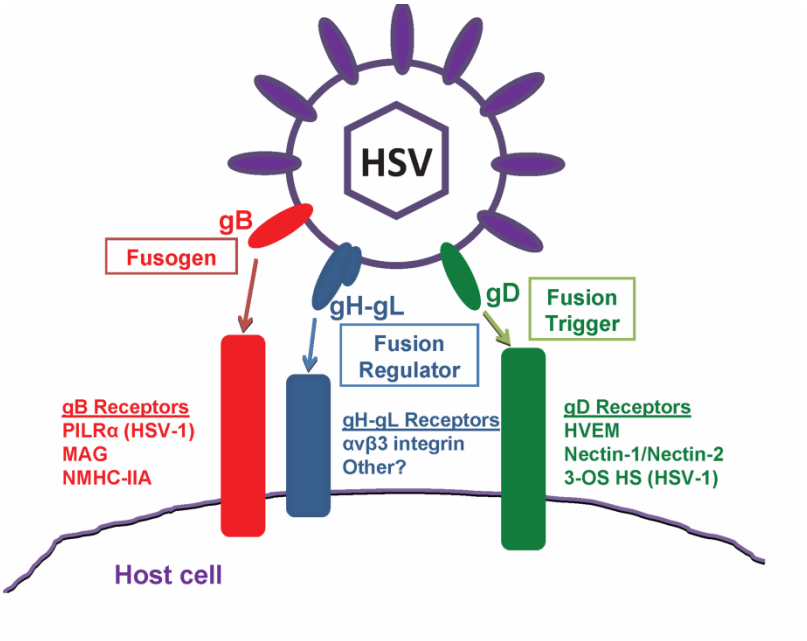


Figure 3. HSV-1 glycoproteins required for viral entry and their identified receptors. HSV-1 entry requires the glycoproteins gB, gD, and the heterodimer gH-gL. Some of the receptors are exclusive to HSV-1 including PILRα and 3-OS HS [52].

The initial contact of HSV-1 with the host cells is the binding of viral gC and/or gB to HS chains of proteoglycans on cell surface [53, 54]. Filopodia, the F-actin-rich membrane protrusions may facilitate the attachment by providing HSPG-rich sites for the initial binding. Although HSV-1 attachment to HS *via* gB and gC enhances the infection, it is not absolutely essential for viral entry [53, 54]. Viral attachment to the cell surface membrane is followed by virus penetration and entry of the nucleocapsid into the cytoplasm. Two major modes of HSV-1 entry exist: 1. a fusion of the virion envelope with the plasma membrane which is pH independent and 2. a generally pH dependent phagocytosis-like endocytosis where the virus triggers the fusion with the membrane of an intracellular vesicle plasma membrane of the host cell [55, 56] (Fig. 4).

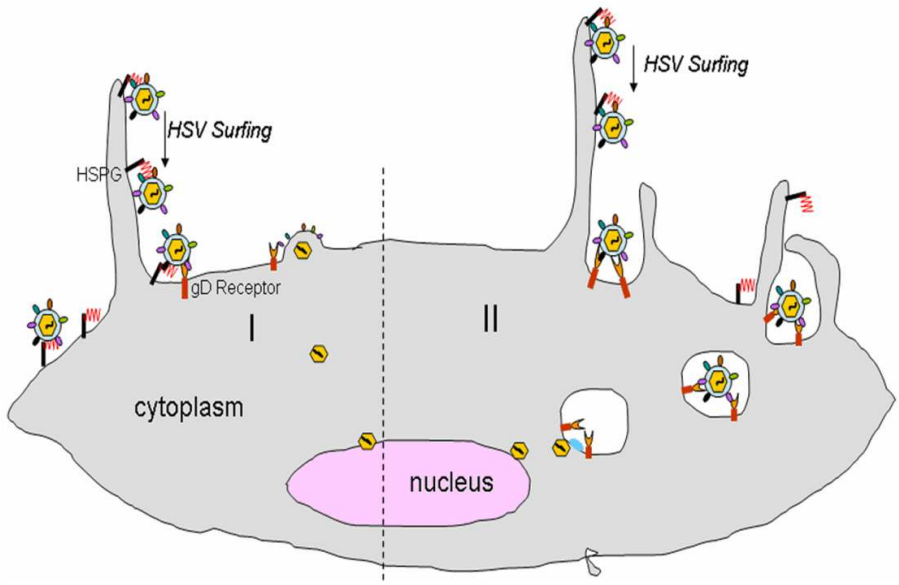


Figure 4. HSV virion and its two major modes of entry into cells. Structural components of a typical HSV virion are shown. HSV virions can enter into cells via a pH-independent fusion of viral envelope with the plasma membrane (I) or, alternatively, via an endocytic pathway that may be phagocytosis-like (II) in terms of the viral uptake [57].

The specific mode of HSV-1 entry takes place in a cell type dependent manner. For example, HeLa and human retinal pigment epithelial cells (RPE) allow HSV-1 entry through pH-dependent endocytic pathway while in African green monkey kidney cells (Vero) HSV-1 can penetrate *via* direct fusion with the plasma membrane at neutral pH [58-60]. Regardless of the entry route followed, HSV-1 enters host cells by inducing fusion between the viral envelope and the host cell membrane and this mechanisim requires essential participation of four viral glycoproteins gB, gD, gH and gL as well as cellular receptors for both gB and gC [61-63]. It has been shown previously that soluble form of gB is capable of binding to HSPG deficient cells, and block virus entry, suggesting the presence of a HS independent gB receptor [64]. Recently, three gB receptors have been identified: 1.) the paired immunoglobulin-like type 2 receptor-α (PILRα) [62], 2.) the myelin-associated glycoprotein (MAG) [63], which are both members of the paired receptor families, and 3.) the non-muscle myosin heavy chain IIA (NMHC-IIA), a subunit of non-muscle myosin IIA [65]. While PILRα and MAG expressions are limited to immune and glial cells, respectively, NMHC-IIA

is ubiquitously expressed in numerous human tissues and cell types, suggesting its important role as the functional HSV-1 gB receptor [52, 65]. Following gC and/or gB interaction with the cell surface, viral entry requires the interaction of gD with one of its entry receptors. These receptors are: nectin-1 (HveC) and nectin-2 (HveB), which are both members of the immunoglobulin superfamily [66], herpesvirus entry mediator (HVEM) that belongs to the tumour necrosis factor receptor family [67], and 3-O-sulfated heparan sulfate (3-OS HS) a specifically modified form of HS [68, 69]. Upon binding of gD to one of its cognate receptors, gD undergoes conformational change, which may transmit an activation signal to gB, and gH/gL leading to membrane fusion. Thus, HSV-1 require essential participation of viral glycoproteins gB, gD, gH and gL which form a multi-glycoprotein complex (fusogenic complex) to execute fusion [56, 70]. Fusion of viral envelope with the cellular membrane results in the release of the naked viral nucleocapsid into the host cytoplasm for transport to the nucleus. HSV-1 has evolved to use multiple receptors and pathways to facilitate entry into different cell types. This differential use of cell membrane receptors is important, and may help in productively infect a wide range of hosts and cell types [71, 72].

1.1.3 Heparan sulfate proteoglycans (HSPGs)

HS is a glycosaminoglycan (GAG) that is present in almost all mammalian tissues on cell surfaces and in the extracellular matrix [73, 74]. HS commonly occurs as part of proteoglycans, where HS GAG chains are attached to a core protein *via* a trisaccharide linkage on a serine residue forming the HSPG [75]. The syndecan family is one of the most abundant HSPGs expressed on mammalian cells [76-78]. There are four members in the syndecan family (syndecan-1, 2, 3 and 4) composed of a single membrane-spanning domain, a conserved transmembrane domain, and an extracellular domain that is specific for each syndecan ([79]. The divergent ectodomains share conserved attachment sites for GAG chains. These GAGs are predominantly heparan sulfated [80, 81] but syndecan-1 and syndecan-4 can also contain chondroitin sulfate (CS) in addition to HS [82, 83]. HSV-1 commonly infects epithelial cells, which express both syndecan-1 and syndecan-2 [84, 85]. In addition, syndecan-1 (CD138, NCBI Reference Sequence: NP_001006947) is also expressed by many other cell types including plasma cells. In contrast, syndecan-2 (fibroglycan, NCBI Reference

Sequence: NP_002989) shows a more restricted expression pattern, limited mainly to fibroblasts and neurons [86-88].

1.2 The role of PRINS non-coding RNA in psoriasis and cellular stress response

1.2.1 Psoriasis

Psoriasis is a frequent, chronic, lifelong inflammatory skin disease, generally characterized by complex alterations in epidermal growth and differentiation. Hyperproliferation of the epidermal cells is believed to be triggered by infiltrating T-lymphocytes at the dermal epidermal junction [89] and by the accumulation of various cytokines and inflammatory mediators which directly modulate keratinocyte proliferation and differentiation [90]. Clinically, psoriasis is a papulosquamous disease with variable morphology, distribution and severity that is characterized by red plaques (due to dilation of blood vessels; raised lesions □1 cm in diameter) with silver or white scales (due to rapid keratinocyte proliferation; raised lesions □1 cm in diameter) that are clearly distinguished from adjacent, normal appearing, non-lesional skin [91]. The morphology of psoriasis can range from small tear shaped papules (guttate psoriasis) to pustules (pustular psoriasis) and generalised erythema and scale (erythrodermic psoriasis). Almost 90% of psoriasis patients have the most common form of the disease, known as psoriasis vulgaris and many affected individuals have a mild form but up to one third of patients have moderate-to-severe form of psoriasis affecting more than 10% of the body surface [92]. Even though cutaneous signs and symptoms are the most common clinical manifestations, nails are frequently affected in up to 50% of cases [93] and approximately 30 % of patients with psoriasis also have joint involvement, indicative psoriatic arthritis [94, 95]. Depending on the severity of disease, psoriasis can be treated with topical agents, ultraviolet light therapy and systemic medications [96].

The exact couse and progress of psoriasis remains poorly understood but it is believed that the combination of genetic and environmental factors contributes to the development of this complex disease [94, 96] and the immune system and its interactive network of leukocytes and cytokines play important role in psoriasis pathogenesis [97, 98].

Environmental causes of psoriasis may include mechanical, ultraviolet and chemical injury of the skin, various infections, prescription drug use, psychological stress and smoking [99-102]. Several studies have revealed that one of the most frequent environmental factors triggering psoriasis is the upper respiratory tract infection caused by β-haemolytic streptococci [103, 104]. Streptococcal infection of the pharynx or tonsils frequently precedes outbreaks of guttate psoriasis in children or, occasionally, adults [105]. According to several studies, psoriasis may not be restricted to the skin; evidence is now emerging of a link between psoriasis and other diseases such as inflammatory bowel diseases (Crohn's disease, colitis ulcerosa) [106], ocular inflammatory diseases (uveitis, iritis) [107], chronic vascular and metabolic disorders (arterial hypertension, hyperlipidemia, diabetes mellitus type II and coronary heart disease) [108], depression, cigarette smoking and heavy consumption of alcohol [109-112].

Family based analyses of patients with psoriasis, especially twin studies; population based epidemiological studies and genome-wide association studies (GWAS) have revealed the strong genetic basis of psoriasis [113-116]. Nine susceptibility loci for psoriasis have been identified using classic GWAS and referred to as, psoriasis susceptibility 1 through 9 (PSORS1 on 6p21.3, PSORS2 on 17q25, PSORS3 on 4q34, PSORS4 on 1q21, PSORS5 on 3q21, PSORS6 on 19p13, PSORS7 on 1p32, PSORS 8 on 16q and PSORS9 on 4q31[117-119]. Altought recent studies have confirmed further susceptibility loci [120-128], the PSORS1 locus is the major susceptibility locus for psoriasis which accounts for approximately 35% - 50% of the genetic risk for this disease [129-131].

Considerable effort has been devoted to identifying genetic factors and biochemical alterations in the epidermis which contribute to the pathogenesis of psoriasis and resulted in the discovery of psoriasis susceptibility loci, a large number of psoriasis-associated genes and aberrantly expressed genes and proteins in the recent decades [125, 132, 133]. A number of gene expression profiling methods like serial analysis of gene expression (SAGE) and microarray analyses have been used in studies to characterize transcriptional changes in psoriasis and identificate large number of novel disease markers [134-139]. These studies were performed on skin biopsies of involved and non-involved, normal appearing psoriatic skin and normal skin from patients with active psorasis and from healthy individuals, respectively. Previous studies have shown that the gene expression pattern of lesional

psoriatic skin is significantly different from normal skin of unaffected individuals [117, 134, 140]. Additionally, the transition from non-involved into involved psoriatic skin is accompanied by changes in the expression of multiple genes [139]. Some of the earliest identified genes as having significant over-expression in involved psoriatic skin include transforming growth factor-α (TGF-α) [141], tumor necrosis factor-α (TNF-α) [142], vascular endothelial growth factor (VEGF) and its receptors [143] and proteinase inhibitors such as peptidase inhibitor 3 (SKALP) [144]. Whereas previous investigations have focused only on the expression of small number of genes [145], recently more comprehensive analyses such as Affymetrix oligonucleotide arrays are used to characterize changes in gene expression of psoriasis [134-136, 139]. Among others, Zhou et al., Romanowska et al. and Gudjonsson et al. have identified a wide range of dysregulated genes in lesional and non-lesional psoriatic skin samples compared with normal skin samples using microarray analyses [136, 139, 140].

Taken together, these microarray studies have identified a number of candidates suggested in original candidate studies as well as novel genes not previously implicated in the pathogenesis of psoriasis. These candidate genes are involved in well-established aspects of psoriasis, such as keratinocyte differentiation, interferon signaling or influx of inflammatory cells and wide range of biological processes are connected with pathogenesis of psoriasis [136, 137]. For istance, fatty acid metabolism; protein trafficking and degradation; cell adhesion; signaling transduction; mitogen-activated responses; RNA binding, regulation and degradation; cytoskeleton restructuring and apoptosis have been correlated with the pathogenesis of psoriasis using microarray analyses [136, 137].

1.2.2 Non-coding RNAs

As our understanding of the complex function of the human genome has advanced, a great role has been ascribed to the enormous part of the genome that does not code proteins. Non-coding RNAs (ncRNAs) have recently been at the centre of attention as a large and diverse group of molecules responsible for a growing list of cellular regulatory functions, such as transcriptional regulation, chromosome replication, RNA processing and modification, messenger RNA (mRNA) stability and translation, protein degradation and translocation [146, 147]. Investigation of the role of ncRNAs in physiological and

pathological mechanisms is therefore of great importance as concerns the elucidation of the complex regulatory pathways involved in human development and disease formation [147].

We earlier performed a differential display experiment to compare the gene expression in the non-lesional psoriatic epidermis and the epidermis from healthy individuals, in order to identify molecular psoriasis susceptibility factors [37]. In that experiment, we identified a novel ncRNA, PRINS which was overexpressed in the non-lesional psoriatic epidermis, and responded with an elevated expression after 12-24 h to various stress signals, including serum starvation, contact inhibition, UV-B irradiation, viral infection (HSV-1) and translational inhibition by cycloheximide in a series of *in vitro* experiments on HaCaT cells [37].

These data indicated that PRINS might play a role in the cellular stress response induced by epidermal stress factors [37]. Knocking down PRINS expression in HaCaT cells by using a vector-based RNA interference method, did not have any impact on the control cells, whereas it decreased the viability of serum-starved keratinocytes, indicating its essential role in the survival of keratinocytes under stress conditions [37]. Furthermore, we have demonstrated that PRINS regulates G1P3, a gene with anti-apoptotic effects in keratinocytes. Our data indicated that the elevated level of PRINS ncRNA in the psoriatic epidermis may contribute to psoriasis by disrupting signal transduction events mediating genes involved in apoptosis regulation such as G1P3 [148].

1.2.3 Nuclear factor-kappaB

NF-κB is a widely studied transcription factor that regulates gene expression in a broad range of cellular processes. The NF-κB system has been shown to play a crucial role in epidermal development and differentiation, stress and immune responses, apoptosis, chronic inflammatory diseases and cancer [149, 150]. It is found in most mammalian cells, and is involved in cellular responses to stimuli of stress such as cytokines, free radicals, UV irradiation, oxidized low-density lipoproteins and bacterial or viral antigens. Within the stratified epidermis, NF-κB is located in the cytoplasm of the proliferative basal layer cells and while keratinocytes are differentiating it shifts to the nucleus in the non-proliferating suprabasal cells [151]. While the molecular mechanisms controlling this process have not been completely identified, change in cellular localization suggests a role for NF-κB in the

progression of keratinocytes from proliferation to growth arrest in the epidermis [151]. Additionally, several *in vivo* studies have suggested a key regulatory role of the NF-κB cascade in growth inhibition control during the process of differentiation of epidermal cells [151, 152]. Currently, five members of the NF-κB/Rel protein family are known: p50/p105, p52/100, c-Rel, p65/RelA and RelB [153]. In the resting state, the dimers are found in the cytoplasm, bound to the inhibitor κB (IκB). Upon stimulation, IκB is phosphorylated by the IκB kinase complex (consisting of IKKα, IKKβ and IKKγ (NEMO)) and subsequently degraded, allowing NF-κB to shift to the nucleus, where it can bind to transcription-regulating elements in a nucleotide sequence-specific manner to activate its target genes [154].

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 Cell lines

Human cervical (HeLa) cells were obtained from B. P. Prabhakar (University of Illinois at Chicago, Chicago, IL, USA). HeLa cells were grown in Dulbecco's modified Eagle's medium (DMEM; Gibco-BRL) supplemented with 10% fetal bovine serum (FBS) and 100 mg of penicillin/streptomycin (P/S). African green monkey kidney cells (Vero), gL-expressing Vero cells (79B4) and Chinese hamster ovarian-K1 (CHO-K1) cells were provided by P. G. Spear (North-Western University, Chicago, IL, USA). Vero and gL-expressing Vero cells (79B4) were grown in DMEM (Gibco-BRL) supplemented with 10% FBS and 100 mg l^{-1} of P/S. CHO-K1 cells were grown in Ham's F-12 medium (Gibco-BRL) supplemented with 10% FBS and P/S. The spontaneously immortalized non-tumorgenic human keratinocytes-derived cell line, HaCaT, kindly provided by Dr. N. E. Fusenig (German Cancer Research Center, Heidelberg, Germany), was cultured in a high-gulcose DMEM (Gibco-BRL) supplemented with 10% FBS, L-glutamine, antibiotics (P/S) and an antimycotic (amphoterecin B; Sigma). HaCaT cells previously stably transformed with an NF-κB responsive element - luciferase reporter gene chimeric construct (NF-κB-HaCaT), kindly provided by Zsuzsanna Györfy (Institute of Biochemistry, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary) were cultured in DMEM/F12 1:1 medium (Life Technologies) supplemented with 10% FBS, L-glutamine and 1% antibiotics (P/S) and an antimycotic solution (amphoterecin B, Sigma - Aldrich). NHKs were isolated from the healthy skin of patients who underwent plastic surgery, using standard protocols, and cultured in keratinocytes serum-free medium (Keratinocyte-SFM; Gibco-BRL) supplemented with antibiotics (P/S) and an antimycotic (amphoterecin B) solution. The medium was changed every 2 days. In our experiments, third-passage NHKs were used at 80% confluence. The study was approved by the Institutional Review Board of the University of Szeged and was performed in adherence to the Declaration of Helsinki Guidelines. Written informed consent was obtained from each patients involved in the study. The approval number form the local ethics committee: Human Investigation Review Board of the University of Szeged, Ref. No.: 2084 issued in April 24, 2006.

3.2 Viruses

The β-galactosidase expressing recombinant HSV-1 (KOS) gL86 and wild-type HSV-1(KOS) viruses were provided by P. G. Spear (North-Western University, Chicago, IL, USA). HSV-1 (KOS) K26GFP was provided by P. Desai (Johns Hopkins University, Baltimore, MD, USA). Jellyfish GFP was fused in-frame with the UL35 ORF generating the K26GFP virus whose capsids express GFP [155]. Virus stocks were propagated in complementing cell lines and stored at –80 ºC. Yields of infectious virus titer (p.f.u. ml^{-1}) were determined by plaque assay in Vero cells.

3.3 Antibodies

Primary antibodies		
	Dilutions	Resources
mouse anti-syndecan-1 mAbs	1 µg per $1x10^6$ cells	Santa Cruz Biotechnology
rat anti-syndecan-2 mAbs	1.25 µg per $1x10^6$ cells	R&D Systems
mouse anti-heparan sulfate mAb (10E4 epitope)	1:50	US Biological
rabbit anti-syndecan-1 pAb	1:500	Santa Cruz Biotechnology
rabbit anti-syndecan-2 pAb	1:500	Santa Cruz Biotechnology
mouse anti-β-actin mAb	1:1000	Sigma-Aldrich
mouse anti-myc mAb	1:500	Life Technologies
Secondary antibodies used for flow cytometry		
FITC-conjugated goat anti-mouse secondary antibody	1:100	Sigma-Aldrich
FITC-conjugated goat anti-rat secondary antibody	1:100	Sigma-Aldrich
FITC-conjugated goat anti-mouse IgM	1:100	Sigma-Aldrich
Secondary antibodies used for Western blot		
HRP-conjugated goat anti-rabbit IgG	1:20000	Jackson ImmunoResearch Laboratories
HRP-conjugated rabbit anti-mouse IgG	1:25000	Jackson ImmunoResearch Laboratories

Table 1. **Antibodies used for flow cytometry and Western blot**

3.4 Enzymes

Heparinase I, II and III enzymes were provided by Jian Liu (University of North Carolina, Chapel Hill, NC, USA) and used at 12 µg per sample (170 µg ml^{-1} final concentration). Chondroitinase ABC was used at 0.005 U per sample (0.1 U ml^{-1} final concentration; Sigma-Aldrich).

3.5 siRNA transfection

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; Sigma-Aldrich), syndecan-2 siRNA (SASI_Hs01_00195372, SASI_Hs01_00195365; Sigma-Aldrich), or a non-specific, scrambled control siRNA (59-GAUCAUACGUGCGAUCAGA[dT][dT]-39; Sigma-Aldrich). Transfection was done by using Lipofectamine 2000 reagent (Life Technologies). To evaluate the efficacy of syndecan-1 and syndecan-2 gene silencing, Western blot analysis of syndecan-1 and syndecan-2 protein expression was performed in a sample of HeLa cells that were mock treated (no transfection) or treated with scrambled siRNA, syndecan-1 siRNA or syndecan-2 siRNA. Transfection efficacy was measured by using a plasmid encoding enhanced GFP (eGFP; Life Technologies) by counting eGFP-positive cells by fluorescence microscopy.

3.6 Plasmid transfection

The plasmids used in the detection of LPS-induced NF-κB activity experiment were pNF-κB-Luc (Stratagene), pGL4.75[hRluc/CMV] (Promega), pSilencer 2.1-U6 hygro (Ambion) and pmaxGFP (Lonza). To knock down PRINS expression, transient transfection experiments using a vector-based RNA interference method [37] were carried out both with the HaCaT cell line stably transformed with the NF-κB-luciferase chimeric construct and with NHKs. Both cell lines were transfected at 80% confluence with 2 μg/well plasmid DNA. Transient transfections were carried out with a Nucleofector device (Lonza) using the appropriate transfection reagents provided for HaCaT cells and 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. Third passage 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). To evaluate the efficiency of PRINS gene silencing, the PRINS RNA level was determined by quantitative real time PCR analysis in transfected NF-κB-HaCaT cells and NHKs. Transfection efficiencies in both NF-κB-HaCaT cells and NHKs were determined

after transfection of plasmid encoding the enhanced green fluorescent protein eGFP (pmaxGFP), using a dual-laser FACS-Calibur flow cytometer (Beckton Dickinson), and analyzed with the CellQuest Software.

3.7 Viral entry assay

Standard entry assay was used as described previously [68]. Briefly, HeLa cells and HSV-1 entry receptor defficient CHO-K1 cells were plated in 96-well tissue culture dish at a density of 8 x 10^3 cells per well. Cells were transfected with syndecan-1, syndecan-2 or scrambled siRNA as described above. After 48 h, cells were infected with the β-galactosidase expressing recombinant HSV-1(KOS) gL86 in a twofold serial dilution for 6 h at 37 ºC. β-galactosidase expression is driven by HSV-1 ICP4 promoter by early viral protein synthesis upon HSV infection [67, 68]. At 6 h post-infection, cells were washed twice with PBS and the soluble substrate O-nitrophenyl-b-D-galactopyranoside (ONPG; Pierce) was added. Enzymic activity was measured at 410 nm using a micro-plate reader (Spectra Max 190 Molecular Devices).

3.8 Antibody blocking assay

Standard antibody blocking assay was used as described previously [156]. Briefly, confluent HeLa cells in 96-well tissue culture dish were washed with PBS and incubated with serial dilutions of rabbit pAbs to sydecan-1, syndecan-2 (Santa Cruz Biotechnology) or control anti-myc mAb (Life Technologies) for 30 min at 37 ºC. A constant dose of HSV-1(KOS) gL86 (m.o.i. of 10) was then added to all wells for 2 h at 37 ºC. After 2 h, cells were washed and the bound viruses were removed by a 20 s treatment with 100 mM citrate buffer (pH 3.0). Incubation was then continued for another 3 h. Cells were then washed twice with PBS and β-galactosidase activity was measured by adding its substrate ONPG as described in virus entry assay.

3.9 Plaque assay

Viral replication upon syndecan-1 and syndecan-2 knockdown was assessed by plaque assay [157]. In brief, monolayers of HeLa cells plated in six-well tissue culture dishes were transfected with syndecan-1, syndecan-2 or scrambled siRNA as described above. At 48 h post-transfection, cells were infected (m.o.i. of 0.01) with HSV-1 (KOS) or mock infected in PBS for 90 min at 37 ºC. Cells were then washed with PBS and fresh medium (DMEM supplemented with 10% FBS and P/S) was added. Cells were incubated for 72 h at 37 ºC. After 72 h, cells were washed with PBS, fixed with 100% methanol for 5 min, and stained with the Giemsa stain (Sigma-Aldrich) for 20 min. Infectivity was assessed by counting plaques formed using a 10x objective lens of an inverted light microscope (Zeiss Axiovert 200).

3.10 Cytotoxicity assay

Confluent monolayer of HeLa cells were transfected with syndecan-1, syndecan-2 or scrambled siRNA as described above. In addition to the non-specific, scrambled siRNA transfected cells, non-transfected cells were also used as controls. Forty-eight hours post-transfection, cells were infected with HSV-1(KOS) (m.o.i. of 0.01) for 90 min at 37 ºC. 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 (version: 1.43) at twenty high power fields (x 40 objective).

3.11 Flow cytometry

Syndecan-1 and syndecan-2 and HS cell surface expression was detected after HSV-1(KOS) infection. For syndecan-1 and syndecan-2 cell surface expression, confluent monolayers of HeLa cells were infected with HSV-1 (KOS) (m.o.i. of 10) for 0, 2, 4 and 6 h. Cells were then washed with PBS, harvested and incubated with the respective primary antibody (syndecan-1 at 1 µg per $1x10^6$ cells, or syndecan-2 at 1.25 µg per $1x10^6$ cells)

diluted in PBS with 1% BSA for 1 h. After primary antibody incubation, cells were washed and incubated for 45 min with anti-mouse or anti-rat-FITC-conjugated secondary anti-IgGs (1:100). Cells stained only with anti-mouse-FITC or anti-rat-FITC were used as background controls. For HS cell surface expression, HeLa cells were infected with HSV-1 (KOS) (m.o.i. of 10) for 0, 2, 4 and 6 h. Cells were then washed with PBS, harvested and incubated with mouse anti-human HS mAb 10E4 diluted 1:50 (US Biological) for 20 min at 4 ºC. After that the cells were washed and incubated for 30 min at 4 ºC with FITC-conjugated anti-mouse IgM diluted to 1:100. Cells stained only with FITC-conjugated anti-mouse IgM were used as background controls. Transfection efficiencies in NF-κB-HaCaT cells and NHKs were determined after transfection of 2 µg/well of pmaxGFP, using a dual-laser FACS-Calibur flow cytometer (Beckton Dickinson) and analyzed with the CellQuest Software.

3.12 Immunoblotting

After 48 h post-transfection of HeLa cells and 0, 2 and 6 h after HSV-1(KOS) infection (m.o.i. of 10), syndecan-1 and syndecan-2 protein expression was determined by Western blot analysis. The Western blot assay was performed according to the protocol described previously [158]. Briefly, approximately 150–200 µg of total cell protein in lysis buffer was incubated with 2.5 volumes of –20 ºC 100% methanol overnight at –20 ºC. After treatment with 500 µl 100% acetone for 5 min, the protein pellet was redissolved in 100 µl 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) twice for 2.5 h to remove all GAGs. Samples were then denatured in Laemmli Sample Buffer (Bio-Rad) with 5% (v/v) 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 for 2 h at room temperature in 0.1% TTBS (0.1% Tween 20 in TBS) containing 5% milk, and incubated with primary rabbit pAbs against sydecan-1 and syndecan-2 at 1:500 dilutions overnight at 4 ºC. The blots were rinsed five times with 0.1% TTBS for 5 min and incubated for 1 h at room temperature with HRP-conjugated anti-rabbit IgG at 1:20000 dilutions. Anti-β-actin mouse mAb as the primary antibody at 1:1000 dilution and HRP-conjugated anti-mouse IgG as the secondary antibody at 1:25000 dilution were used for detecting β-actin as a

loading control. The signal was visualized with SuperSignal West Femto maximum sensitivity substrate (Pierce) and the blots were exposed to X-ray film (Kodak) for 2 min. Developed films were scanned and protein bands were quantified using the NIH Image J software (version: 1.42) in order to generate statistical data for specific bands. Syndecan-1 and syndecan-2 protein expression was quantified by calculating the relative intensity of each syndecan-1 and syndecan-2 band relative to the bands of mock-treated cells.

3.13 Detection of LPS-induced NF-κB activity

Luciferase assays were performed to determine the NF-κB activity in response to LPS (purified from *Escherichia coli*; Sigma-Aldrich) 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 (HaCaT cells previously stably transformed with the NF-nB/luc/neo reporter plasmid, [159]) 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. 24 h after transfection, cells were incubated with 2.5 μg/ml LPS for 4 h. The treated cells were washed twice with PBS and lysed with passive lysis buffer (Promega) and the luciferase activities in the lysates were measured with the Promega Luciferase Assay System according to the manufacturer's instructions (Promega). Firefly and Renilla luciferase activities were measured in a Thermo Luminoskan Ascent Machine (Thermo Scientific). For the standardization of transfection efficiency in NHKs, the luciferase activity derived from pNF-κB-Luc was normalized to the activity of Renilla luciferase. The protein concentrations of NF-κB-HaCaT cell lysates were determined by using the Bradford protein assay. Transient transfection efficacy was detected with positive control vector pmaxGFP by FACS analysis. The efficacy of PRINS silencing was detected by using real-time RT-PCR for each experiment. Results were confirmed by at least three independent transfections.

3.14 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 (Bio-Rad). 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. TaqMan probes were purchased from IDT Probes. The RT-PCR reactions were performed with the iQ Supermix (Bio-Rad) in an iCycler (Bio-Rad). The primer sets and TaqMan probes used for the reactions are listed in Table 2.

	Forward primer	Reverse primer	Probe
PRINS	GCATCTTCCCTTGGCAAA	GCCTAAAGGACATTTCGGTAT	TGC TGT TTT GGG TCC TAA CCA TC
18S	CGGCTACCACATCCAAGGAA	GCTGGAATTACCGCGGCT	TGC TGG CAC CAG ACT TGC CCT C

Table 2. Sequence of primers and probes used for RT-PCR.

3.15 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. *$p<0.05$ and **$p<0.0001$ were regarded as significant differences between treated and mock-treated groups. The results are expressed as means ± SD values; each experiment was repeated at least three times.

To evaluate the effects of silencing of PRINS on LPS-induced NF-κB activity data were assessed using the repeated measures ANOVA. *$p < 0.05$ was regarded as significant differences between treated and mock-treated groups. The results are expressed as mean ± (SE) values; each experiment was repeated at least three times.

4. RESULTS

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

Earlier reports have established that infection with microbial pathogens can result in significant changes in syndecan expression [33-35, 160]. 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. As shown in Figure 5., 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.

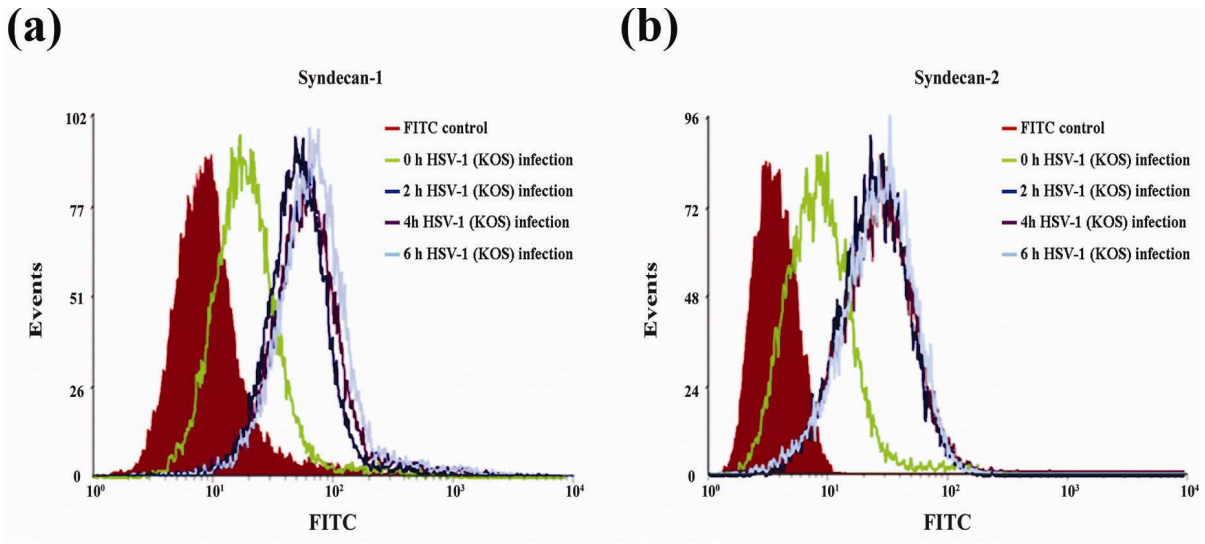
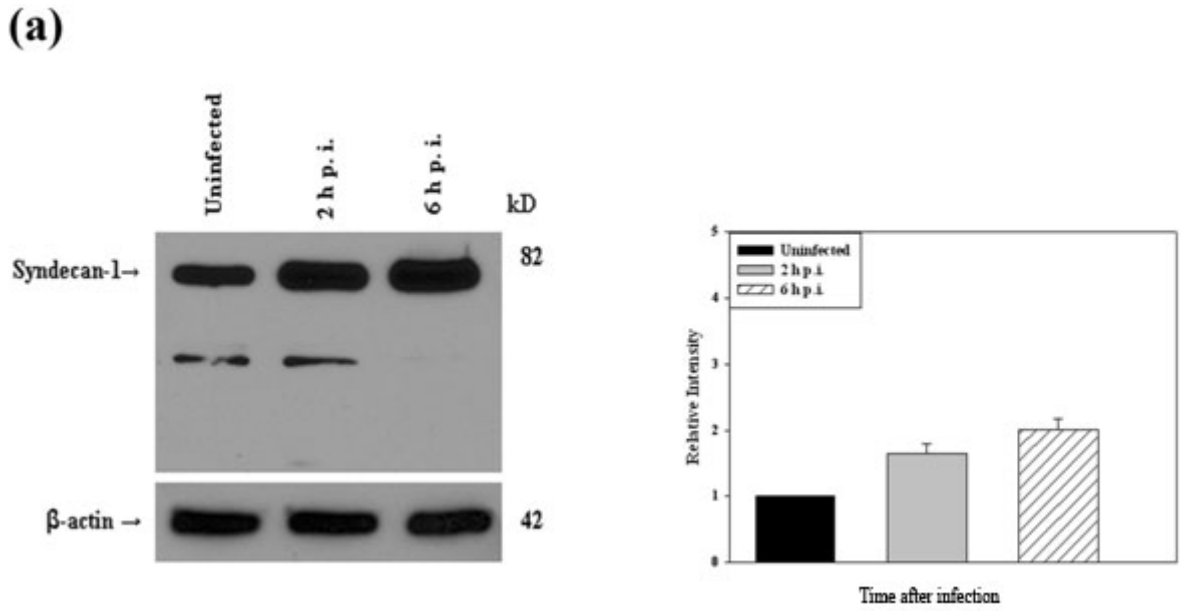


Figure 5. HSV-1 infection of host cell enhances syndecan-1 and syndecan-2 cell surface expression in HeLa cells. Cells were infected with a constant dose of HSV-1 (KOS) (m.o.i. of 10) for 2, 4 or 6 h. Syndecan-1 and syndecan-2 cell surface expression was then detected by FACS analysis. Enhanced syndecan-1 (a) and syndecan-

2 (b) cell surface expression was detected in HeLa cells following HSV-1 infection. Mock-infected FITC stained cells were used as background control. Results are representative of three independent experiments.

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 (Fig. 6a). 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 infection (Fig. 6b). 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.



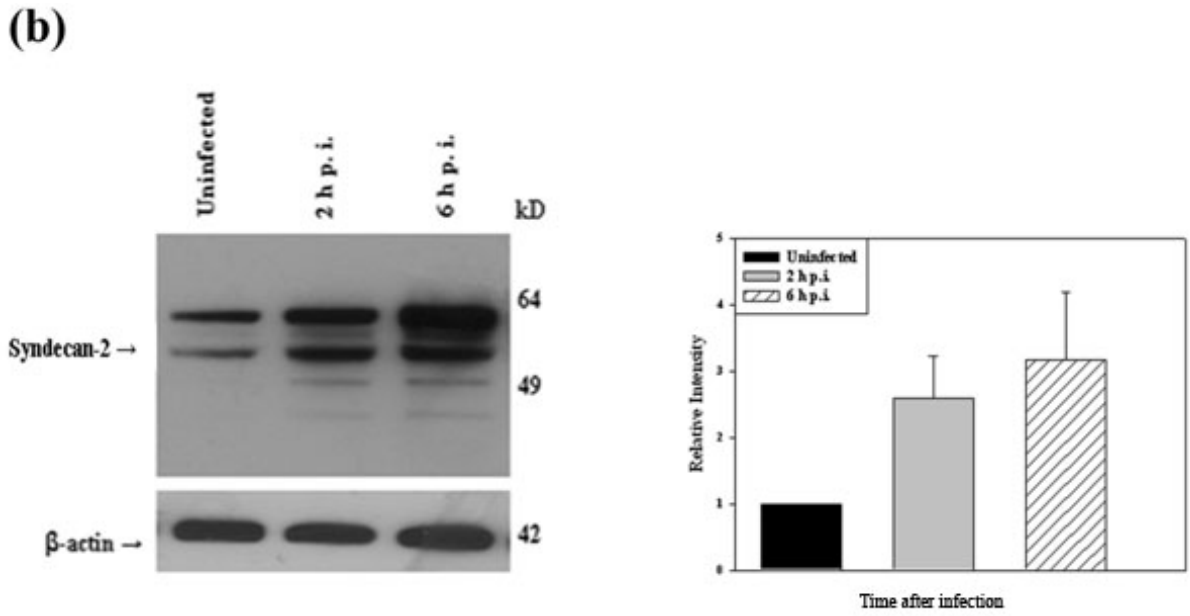


Figure 6. Western blot analysis of syndecan-1 and syndecan-2 protein expression after HSV-1 (KOS) infection. HeLa cells were infected with a constant dose of HSV-1 (KOS) (m.o.i. of 10) for 2 and 6 h. Cell lysates were then probed for syndecan-1 and syndecan-2 expression. According to the densitometric analysis, syndecan-1 (a) and syndecan-2 (b) were expressed at a higher level at 2 and 6 h post-infection when compared to uninfected HeLa cells. Relative intensity of HSV-1(KOS) virus-infected bands expressed as a ratio relative to the mock-infected sample is shown. β-actin protein expression was measured as loading control. (means ± SD from three independent experiments).

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 (Fig. 7). 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.

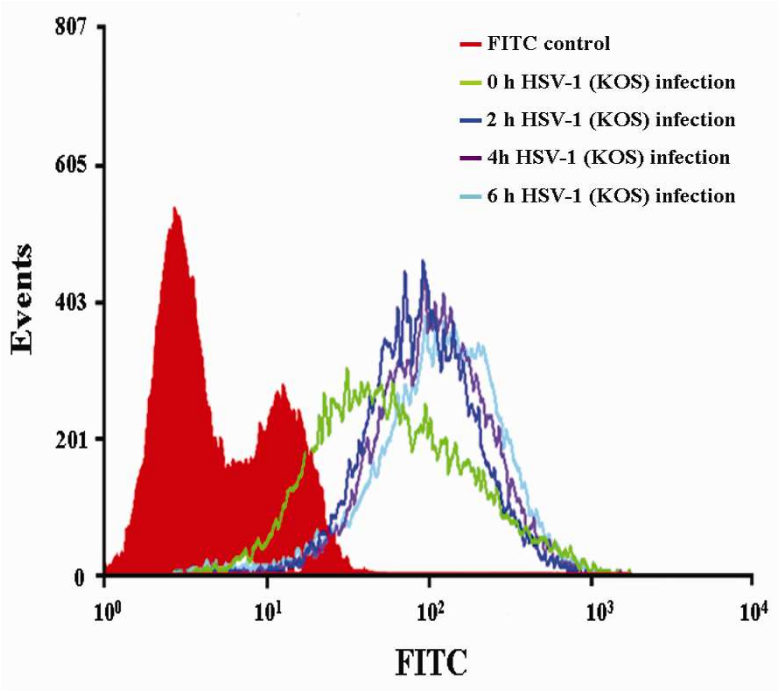


Figure 7. Flow cytometry analysis of HS expression on HeLa cells infected with HSV-1. HeLa cells were infected with HSV-1(KOS) (m.o.i. of 10) for 2, 4 or 6 h. HS cell surface expression was then detected by FACS analysis. In HSV-1-infected cells, HS cell surface expression was enhanced compared with uninfected cells. Untreated FITC-stained HeLa cells were used as background control. Results are representative of three independent experiments.

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 [79]. Cells were treated with either syndecan-1- or syndecan-2-specific siRNA to downregulate syndecan-1 and syndecan-2 gene expression, respectively. Gene silencing of syndecan-1 (Fig. 8a) and syndecan-2 (Fig. 8b) 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 (about 50%) reduction in syndecan-1 and syndecan-2 protein expression, respectively. The effect of siRNA was specific since scrambled siRNA failed to bring down syndecan expression and likewise,

neither scrambled nor syndecan-specific siRNAs had any effects on β-actin expression. Additional experiments (data not shown) demonstrated that siRNA against syndecan-1 was specific to its subtype and did not interfere with the expression of syndecan-2 and *vice versa* syndecan-2 siRNA also demonstrated high specificity.

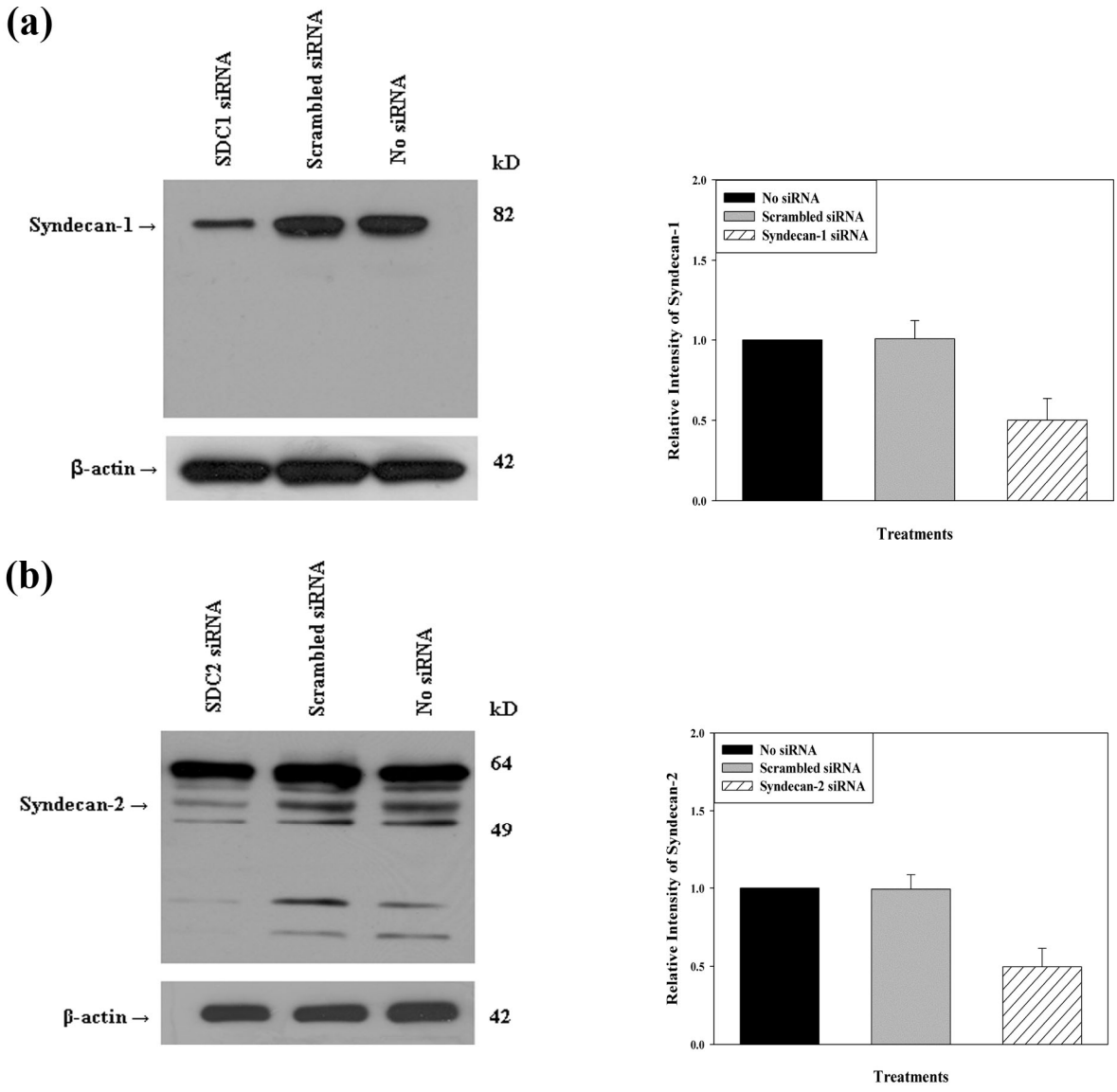


Figure 8. Western blot analysis of syndecan-1 and syndecan-2 protein expression after siRNA downregulation. Protein expression of syndecan-1 and syndecan-2 measured in a sample of HeLa cells mock treated (no transfection) or treated with scrambled siRNA, syndecan-1 siRNA or syndecan-2 siRNA for 48 h. Representative Western blots showed knockdown of syndecan-1 (a) or syndecan-2 (b) after siRNA downregulation. Densitometric analysis revealed 50% reduction in the signal intensity of both syndecan-1 and

syndecan-2. β-actin protein expression was measured as a loading control. (means ± SD from three independent experiments).

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

After verifying syndecan-1 and syndecan-2 downregulation by siRNA transfection, the effect of reduced syndecan-1 and syndecan-2 protein levels on HSV-1 entry into HeLa cells was examined. A previously described HSV-1 entry assay [71, 157] was used to compare viral entry into cells treated with syndecan-1 or syndecan-2 siRNA with those treated with scrambled siRNA or mock treated. HeLa cells were infected with a recombinant β-galactosidase expressing HSV-1 (KOS) gL86 reporter virus. The entry of HSV-1 was measured after 6 h of viral infection. As indicated in Fig. 9a, 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 ($p<0.0001$) (Fig. 9b).

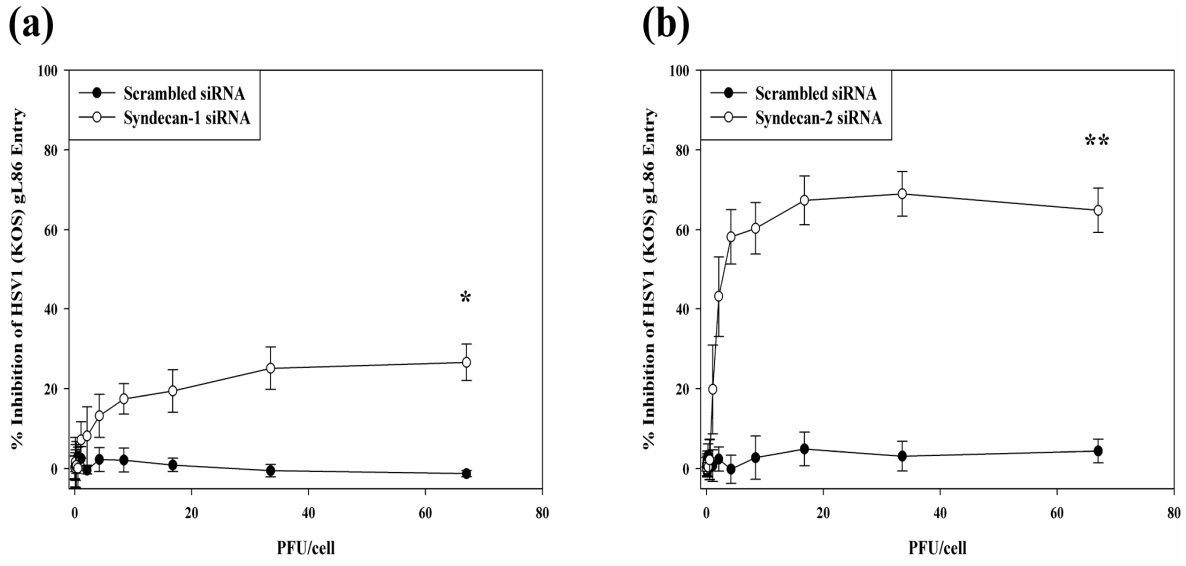


Figure 9. Downregulation of syndecan-1 and syndecan-2 inhibits HSV-1 entry into HeLa cells. HSV-1 entry was analysed in HeLa cells mock treated (no transfection) or transfected with scrambled siRNA, syndecan-1 siRNA or syndecan-2 siRNA. After siRNA transfection, cells were inoculated with a serial dilution of β-

galactosidase expressing recombinant HSV-1 (KOS) gL86 virus for 6 h. The soluble substrate ONPG was added and enzymic activity was measured. Downregulation of both syndecan-1 (Fig. 8a) and syndecan-2 (Fig. 8b) inhibits HSV-1 (KOS) gL86 entry into HeLa cells although syndecan-2 siRNA has more significant effect. Percentage of inhibition of HSV-1(KOS) gL86 entry by syndecan-1 siRNA or syndecan-2 siRNA treatment was calculated relative to mock-treated (no transfection) cells. Scrambled siRNA-transfected cells were used as negative control. (means ± SD from six independent experiments; *$p<0.05$, **$p<0.0001$).

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

To confirm the role of syndecan-1 and syndecan-2 in HSV-1 entry and to determine whether reduced HSV-1 entry after syndecan-1 and syndecan-2 knockdown is specific to syndecan ectodomains, a previously described antibody blocking assay [156] and pAbs blocking syndecan-1 and syndecan-2 ectodomains were utilized. As shown in Figure. 10, 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. Although both syndecan-1 and syndecan-2 pAbs reduced HSV-1 entry, syndecan-2 pAbs blockade had a statistically significant reducing effect ($p<0.05$) that was more substantial than that caused by syndecan-1 pAbs treatment. The effect of the pAbs was not additive, since a cocktail containing both antibodies did not result in a stronger inhibition beyond what was seen with syndecan-2 pAbs (data not shown).

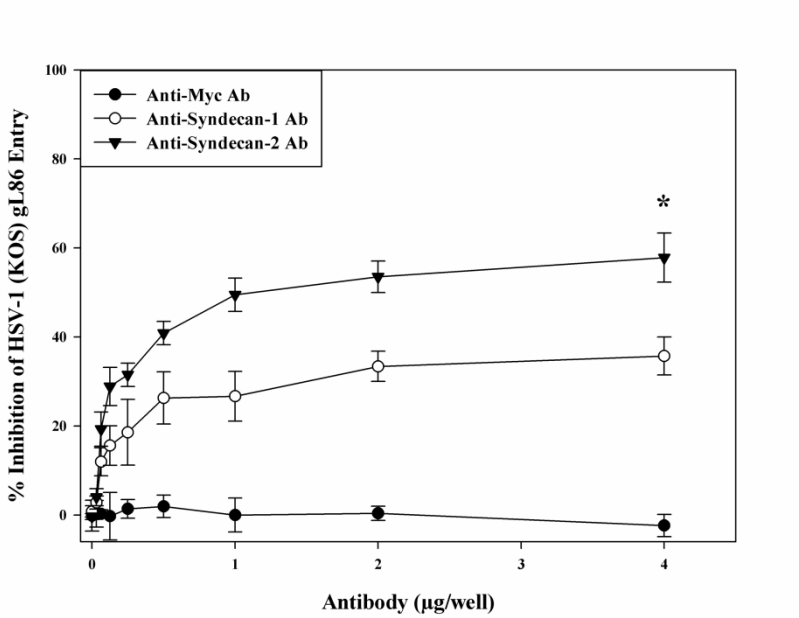


Figure 10. Syndecan-1- or syndecan-2-specific antibody treatment blocked HSV-1(KOS) gL86 entry into HeLa cells. Cells were incubated with a serial dilution of syndecan-1-specific pAbs, syndecan-2-specific pAbs, or control anti-myc mAb for 30 min. Cells were then inoculated with a constant dose of β-galactosidase-expressing recombinant HSV-1(KOS) gL86 (m.o.i. of 10) for 2 h. The soluble substrate ONPG was added and enzymic activity was measured. Per cent inhibition of HSV-1(KOS) gL86 entry by syndecan-1 pAbs or syndecan-2 pAbs treatment was calculated relative to anti-myc mAb-treated cells. (means ± SD from four independent experiments; *$p<0.05$)

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. Since the downregulation of syndecan-1 and syndecan-2 reduced HSV-1 entry, we wanted to determine whether a similar decrease in HSV-1 plaque formation and spread in HeLa cells is also observed after syndecan-1 and syndecan-2 downregulation by siRNA transfection. To this end, a standard plaque assay was performed [157]. As shown in Figure 11a, there was a clear and significant reduction in the number of plaques formed in HeLa cells transfected with syndecan-1- and syndecan-2-

specific siRNAs compared with mock treated or scrambled siRNA-transfected cells. Transfection with syndecan-1-specific siRNA reduced plaque number by 63.22 ± 2.65% ($p<0.0001$), while transfection with syndecan-2-specific siRNA reduced plaque number by 98.73 ± 5.78% ($p<0.0001$). Images taken of formed plaques show that the downregulation of syndecan-1 and syndecan-2 resulted in the formation of smaller plaques compared to those of mock-treated or scrambled siRNA-treated cells (Fig. 11b).

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. As shown in Figure 10c, 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 %, $p<0.05$).

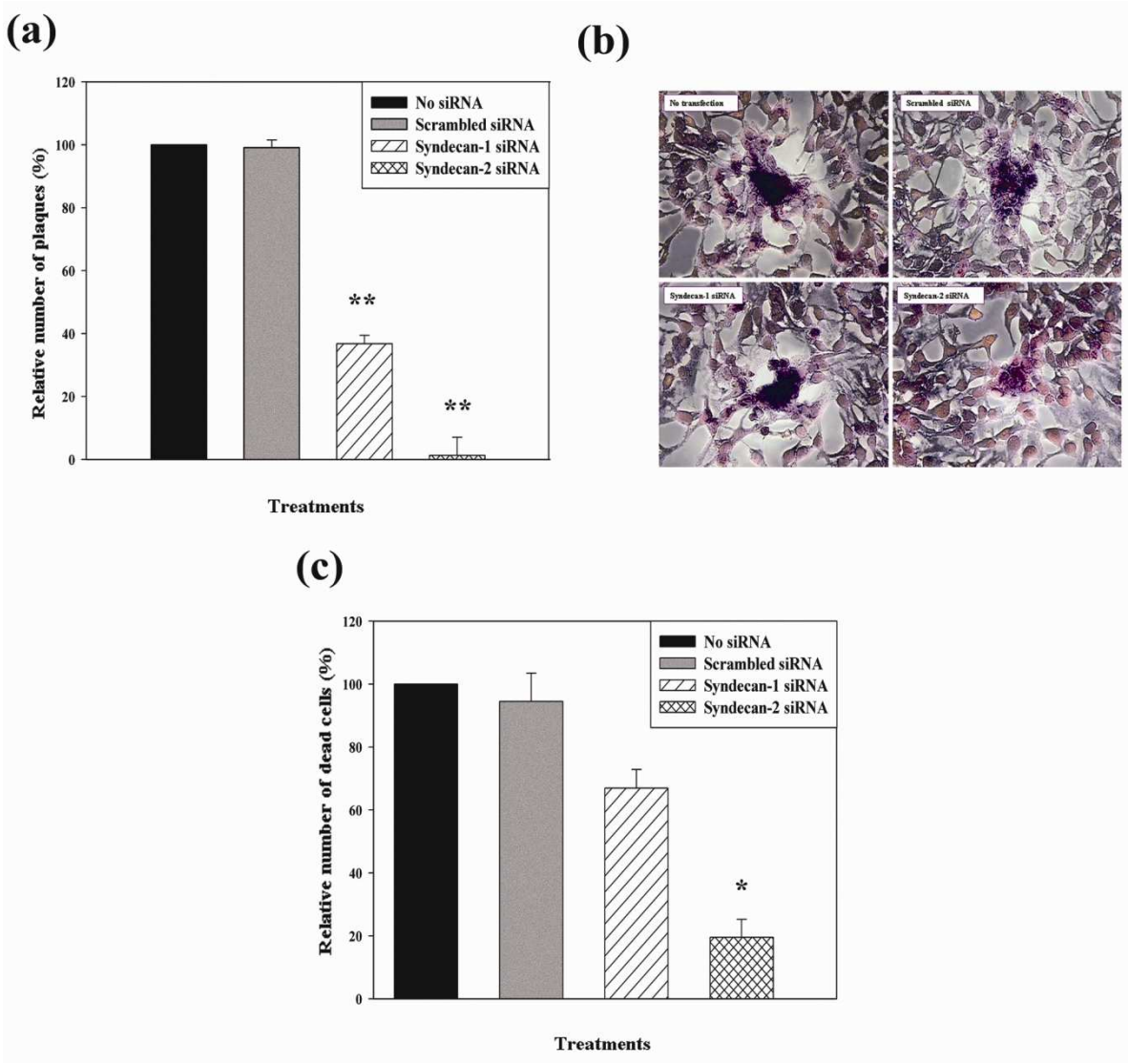


Figure 11. Downregulation of syndecan-1 and syndecan-2 affects HSV-1 plaque formation, size and enhances cell survival in HeLa cells. Cells were either mock treated (no siRNA transfection), or treated with scrambled siRNA, syndecan-1 siRNA or syndecan-2 siRNA. Cells 48 h post-transfection were infected with HSV-1(KOS) (m.o.i. of 0.01). (a) Post-infection infectivity at 72 h was assessed by counting p.f.u. Relative number of plaques was computed relative to mock-treated (no siRNA transfection) samples. Significant decreases in number of plaques were seen in both syndecan-1 siRNA- and syndecan-2 siRNA-transfected HeLa cells. Plaques that consisted of 15 or more nuclei were counted. (means±SD from four independent experiments conducted in triplicate; **$p<0.0001$) (b) Morphological appearance of Giemsa-stained HSV-1 (KOS) plaques 72 h post-infection. In syndecan-1 siRNA- and syndecan-2 siRNA-treated HeLa cells smaller plaques were observed compared to the plaques in mock-treated or scrambled siRNA-treated cells. Magnification, x40. (c) Cytotoxicity was measured 120 h after HSV-1 (KOS) infection. Relative number of dead cells was calculated

relative to mock-treated (no siRNA) samples. Significant decreases of cytotoxicity were observed in both syndecan-1 siRNA- and syndecan-2 siRNA treated cells; however, only the effect of syndecan-2 siRNA treatment was statistically significant. (means ± SD from four independent experiments conducted in triplicate; *$p<0.05$).

4.8 PRINS expression is regulated differentially in NHKs and HaCaT cells upon stess induction

In another set of experiments we aimed to investigate the role of the PRINS non-coding RNA in LPS-induced stress response of keratinocytes. These experiments were carried out using both NHKs and an immortalized keratinocyte cell line, the HaCaT. To this end we first compared the PRINS expression of these two cell types upon various stress inductions. As shown previously, 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 all induced marked PRINS expression in HaCaT cells, in NHKs the same stressors either induced PRINS with a differential pattern (translation inhibition with cycloheximide and UV-B irradiation) or did not induce PRINS expression (microbial compounds) [38]. These data indicate a differential role for PRINS in the cellular stress response in NHKs and in the immortalized HaCaT cells.

These gene expression experiments were performed by my coauthor, Dr. Lilla Bari and will be presented in detail in her PhD thesis.

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

A number of reports have indicated that immortalized HaCaT cells and NHKs exhibit differences in stress response, partially due to the aberrant NF-κB activity seen in HaCaT cells [161]. 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. Since NF-κB activation is considered an end-point of signal transduction processes of cellular stress

response, we applied an experimental set-up in which the effect of PRINS silencing on LPS-induced NF-κB activation could be studied both in HaCaT cells and in NHKs.

Silencing of PRINS gene expression in NF-κB-HaCaT cells and in NHKs transfected with the AK696 PRINS specific silencing construct assessed by real-time RT-PCR, indicated effective silencing, to the extent of 70.66 ± 17.47% and 72.05 ± 22.78%, respectively as compared with cells transfected with the control SC1313 construct shown in Figure 12.

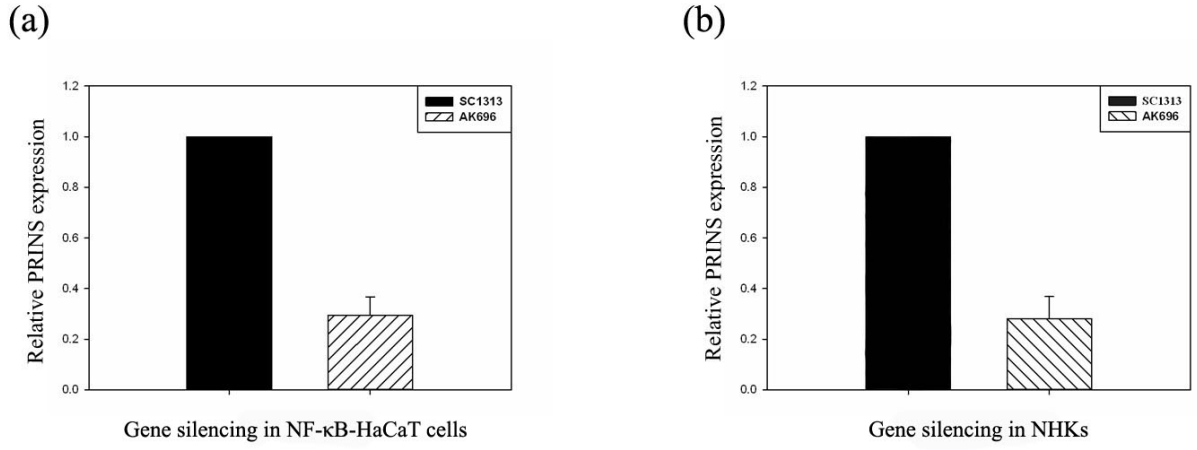


Figure 12. Downregulation of PRINS ncRNA using the AK696 PRINS-specific silencing construct in NF-κB-HaCaT cells and NHKs. (a) NF-κB-HaCaT cells were transfected with the PRINS silencing pSilencer 2.1-U6 construct (AK696) and a construct harboring a scrambled sequence of PRINS gene (SC1313) and (b) NHKs 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). PRINS expression was determined by reverse transcriptase real time PCR analysis. Data are indicated as fold expression compared with the controls (SC1313). (means ± SD from three independent experiments).

Our results indicated that gene-specific PRINS silencing did not affect the LPS-induced NF κB activity either in HaCaT cells (Fig. 13a) or in NHKs (Fig. 13b). In HaCaT cells, we saw no change in NF-κB signal in transfected cells, while in NHKs a non-specific effect was seen in both the control and the specific silencing construct-transfected cells. 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. This

suggests that PRINS is not an upstream effector of NF-κB signalling in keratinocytes, and that the observed differences in stress-induced PRINS expression in HaCaT cells and in NHKs are not related to the aberrant NF-κB activity of HaCaT cells. We therefore hypothesize that PRINS may signal independently of NF-κB, and another novel cellular processes may lie behind the differences in stress-induced PRINS expression between the two cell types.

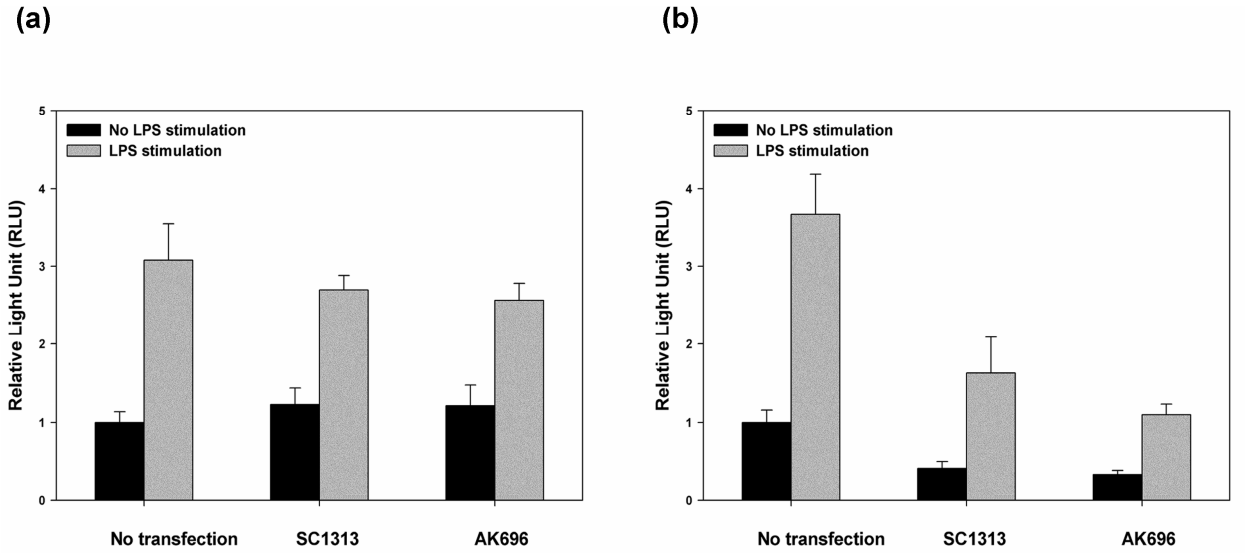


Figure 13. The effects of PRINS silencing on LPS-induced NF-κB activity in HaCaT cells and in NHKs. Luciferase assays were performed to assess NF-κB activity in response to LPS stimulation in NF-κB-HaCaT cells (a) and in NHKs (b). Silencing of PRINS had no effect on the LPS-induced NF-κB activity either in HaCaT cells or in NHKs.

5. DISCUSSION

Epithelial cells compose the covering of most internal and external surfaces of the human body including the outer layer of the skin (epidermis), as well as the surface of most body cavities including the respiratory, the gastrointestinal, the reproductive and the urinary tract. The surface of exocrine and endocrine glands is also composed by epithelial cells. Epithelial cells constitute the first defense line against invading pathogens and have a proactive role in immune responses and the development of localized inflammatory conditions. To protect the human body and respond to stress conditions caused by microbial infection, epithelial tissue apply three main strategies. Firstly, epithelial cells form an impermeable physical barrier that prevents pathogen entry; secondly epithelial cells are able to produce defense molecules including antimicrobial peptides and proteinase inhibitors; finally these cells can produce and release signaling molecules including lipid mediators, grow factors, and variety of cytokines/chemokines [162, 163]. These signaling molecules activate cells of the innate and adaptive immune system [164].

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 [51, 165]. HS can serve as a receptor for a wide range of microbial pathogens, including viruses and bacteria [166-168]. 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, [84] 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.

Previous studies have shown that the infection with various microbial pathogens can modulate the expression levels of different syndecan family members. For example, alterations in syndecan-1 expression level are observed during the infection with *Pneumocystis jiroveci* and *Neisseria gonorrhoeae* [34, 35]. Epstein–Barr virus infection results in the downregulation of syndecan-1 [33]. Our study is the first report of HSV-1 infection modulating the expression levels of syndecan-1 and syndecan-2 in infected cells. We demonstrated that HSV-1-induced syndecan-1 and syndecan-2 expression enhancements occur 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 a 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 [157, 169]. 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. For unclear reasons, the syndecan pAbs when combined together did not produce an additive effect. Future studies will determine whether the syndecan pAbs (especially syndecan-2 pAbs) may cross-react to block certain conserved syndecan epitopes shared for entry. In this case, one group of pAbs (e.g. syndecan-2 pAbs) may be able to block all the epitopes (whether on syndecan-1 or syndecan-2) and therefore, pAbs to another protein may not be able to show any additional effects. 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. However, high affinity interactions (accompanied by conformation

changes) may not be blocked by antibodies and therefore, the net effect by combining the antibodies may not be significantly higher than the individual effects. This may be a reason why a near complete blocking of HSV-1 infection by antibodies has been extremely rare even when all gD receptors were blocked [157, 169]. 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 [170]. 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 [83, 88, 171]. 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. A second way to explain the observable differences relies on HSPG cytoplasmic domain that has been shown to interact with a variety of signalling and structural proteins, suggesting its involvement in various regulatory phenomena. The cytoplasmic domain consists of two conserved regions, a membrane proximal common region (C1) and C-terminal common region (C2). C2 mediates binding to cytoskeletal proteins and to PDZ-containing proteins [81, 172, 173]. The C1 and C2 conserved regions are separated by a variable region (V) that is unique for each of the four syndecan family members [81, 172]. The difference in the variable region in syndecan-1 and syndecan-2 might explain how these two syndecans might have different regulatory roles during HSV-1 infection through activating different cellular pathways. 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 [37]. PRINS, localized on the short arm of human chromosome 10, consists of two exons, and its full length transcript, expressed by the immortalized keratinocytes cell line HaCaT, is 3.6 kb long [37]. PRINS harbours two *Alu* repetitive sequences, and also contains a heat-shock element that displays approximately 70% similarity to G8, a small ncRNA in *Tetrahymena thermophila* [37]. 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 [37]. 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 [37]. Gene-specific silencing of PRINS led to a decreased viability of serum-starved HaCaT keratinocytes as opposed to the control cells, where the silencing of PRINS under the normal culturing conditions did not have an impact on cellular viability [37]. These data led us to hypothesize that PRINS is essential for the survival of keratinocytes under stress conditions.

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 [38]. We found that PRINS expression responded differentially to various stress signals and microbial agents in HaCaT cells and in NHKs.

Epidermotropic viruses exert a series of structural and biochemical changes in invaded host cells, one of which is translational inhibition. Incubation of HaCaT cells and NHKs with cycloheximide, a chemical inhibitor of mRNA translation, resulted in elevations of PRINS expression of similar magnitude, but with different time courses in the two cell types [38]. 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 [38]. It is well documented that distinct differences exist between the responses of NHKs and HaCaT cells to UV-B irradiation and HaCaT cells are known to be more sensitive to UV-B-induced apoptosis [174]. However, 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, but none of the other microbial agents [38].

Under normal conditions, the epidermis is subjected to a wide range of pathogenic and non-pathogenic microbes, which can activate the innate immune system through a variety of pattern recognition receptors, such as the family of TLRs. It is well established that signaling events induced by LPS stimulation are mediated through TLR 4 [175], while yeast and Gram-positive bacterial and mycobacterial components bind to TLR 2 [176]. The effect of LPS is mediated through TLR4 in keratinocytes. Keratinocytes also express TLR2, a receptor to yeast and Gram-positive bacterial and mycobacterial components [177]. Olariu et al. previously demonstrated that HaCaT cells and NHKs expressed similar levels of TLR2 and TLR4 mRNAs, and that HaCaT cells stimulated with various microbial agents responded with the induction of various chemokine mRNAs similarly to NHKs [178]. Taken together, these data suggest that, although HaCaT cells exhibit differences in some applications i.e. regulation of tissue differentiation [179], they are suitable tools for studying certain aspects of keratinocyte functions. 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. The results of our previous work [180] suggested that

PRINS may primarily contribute to cellular events regulating keratinocyte apoptosis, since we found that PRINS regulated the expression of G1P3, an anti-apoptotic gene that is under the control of the JAK/STAT/ISRE signal transduction pathway [181]. Overall, our data indicate that PRINS, a novel ncRNA related to the cellular stress response [37], signals independently of the NF-κB-mediated signal transduction pathway(s). Moreover, our results suggest that there may be differences in intracellular signaling pathways between NHKs and HaCaT cells and this is also reflected in the differences in PRINS expression seen in these two cell types.

The identification of genes with altered expression patterns either in involved or in non-involved psoriatic skin widely contributes to the better understanding of the pathogenesis of psoriasis. Previous studies have been revealed large scale gene expression changes in involved psoriatic skin compared to normal skin [134, 136, 137]; involved psoriatic skin compared to non-involved psoriatic skin [136, 137, 140]; and non-involved psoriatic skin compared to normal skin [139]. While numerous psoriasis-associated genes have been identified [117, 134, 137, 139, 140], the role of these genes in the pathogenesis of psoriasis remains poorly understood. Previously, we identified a novel non-coding RNA, PRINS that is expressed at higher level in the non-involved epidermis of psoriatic patients than in either the psoriatic involved epidermis or epidermis of healthy individuals [37]. Our results suggest that the overexpression of PRINS in the non-involved psoriatic epidermis may play role in psoriasis susceptibility [37] and are in accordance with previous studies that in response to external stress stimuli, the keratinocytes of the non-involved psoriatic and of the healthy epidermis answer differentially [182, 183]. We additionally showed that the expression of the anti-apoptotic G1P3 gene is under the contol of PRINS [148] and PRINS might interact physically with the molecular chaperone protein nucleophosmin in keratinocytes [148]. We suppose that PRINS is part of a ribonucleocomplex and its altered expression in psoriatic uninvolved epidermis contributes to the well-established aberrant stress response of psoriatic keratinocytes and as a consequence to psoriasis susceptibility.

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.

6. SUMMARY

In our work, we investigated two aspects of epithelial cell stress responses following viral and bacterial infection. In the first part of our work, we examined the role of two heparan sulfate proteoglycans on HSV-1 infection and in the second part of our work we examined the connection between the NF-κB and PRINS-mediated signal transduction pathway in NHKs and HaCaT cells.

Herpes simplex virus type 1 (HSV-1) is an important human pathogen and a leading cause of infectious blindness in the developing world. HSV-1 exploits heparan sulfate proteoglycans (HSPG) for attachment to cells. While the significance of heparan sulphate (HS) moieties in HSV-1 infection is well established, the role of specific proteoglycan core proteins in the infection process remains poorly understood. The objective of this study was to assess the roles of syndecan-1 and syndecan-2 core proteins in HSV-1 infection, both of which are expressed by many HSV-1 target cell types. HSV-1 infection increases syndecan-1 and syndecan-2 protein synthesis and results increase in cell surface expression of HS. Furthermore, our results demonstrate that syndecan-1 and syndecan-2 gene silencing by RNA interference reduces HSV-1 entry, plaque formation and facilitates cell survival. Our observations suggest that changes in syndecan-1 and syndecan-2 expression levels may be related to active viral infection. Taken together, our findings provide new insights into HSPG functions during HSV-1 entry and spread.

Psoriasis is a chronic inflammatory skin disease that affects approximately 2–4% of the population. We recently described a novel non-coding RNA, psoriasis susceptibility related RNA gene induced by stress (PRINS), that was overexpressed in non-lesional psoriatic epidermis, and its expression was induced by various stress factors such as serum starvation, contact inhibition, ultraviolet (UV)-B irradiation, viral infection and translational inhibition in HaCaT cells. We compared the stress and microbial agent-induced PRINS expression in normal human keratinocytes (NHKs) and HaCaT cells and we observed different PRINS expression pattern responded to various stress signals and microbial agents in these two cell lines. To explore whether the known NF-κB abnormalities in HaCaT cells could be related to this differential PRINS expression, we silenced the PRINS gene expression with small

interfering RNA (siRNA) in both HaCaT cells and in NHKs and monitored NF-κB signal transduction after lipopolysaccharide (LPS) treatment. Silencing of PRINS had no effect on LPS-induced NF-κB activity either in HaCaT cells or in NHKs. Our results indicate that PRINS probably affects keratinocytes functions independently of NF-κB signalling.

7. ÖSSZEFOGLALÁS

Munkánk célja a virális és a bakteriális fertőzést követő sejtszintű stressz válaszok tanulmányozása volt epithél sejtekben. Munkánk első részében két, a herpes simplex virus-1 (HSV-1) fertőzésre fogékony sejtek felszínén általánosan kifejezett sejtfelszíni heparán-szulfát proteoglikán (HSPG), a syndecan-1 és syndecan-2 szerepét tanulmányoztuk a HSV-1 fertőzésben human epithél sejtekben (HeLa), míg munkánk második részét az NF-κB és PRINS-közvetített jelátviteli útvonal közti kapcsolat vizsgálata képezte primer keratinocitákban és HaCaT -sejtekben.

A herpes simplex-1 világszerte elterjedt patogén vírus. A HSV-1 által leggyakrabban okozott betegségek a bőr felhólyagosodásával járó ajak-herpesz; a vakságot is okozható szaruhártyaherpesz, de ritka esetekben a lappangó HSV-1 vírus aktivációja akár encephalitist (herpes-encephalitis) is előidézhet. A herpeszfertőzés a sérült bőr vagy nyálkahártya epithél sejtjeiben megy végbe. Az elsődleges fertőzést követően a HSV-1az érzőideg-végződésekbe jut, majd az idegnyúlványokon keresztül a gerincvelő hátsó gyöki ganglionjaiban telepszik meg, és lappang élethossziglan. A szervezetben lappangó herpeszvírusok reaktiválódhatnak különböző tényezők hatására, úgymint immunszupresszió, láz, hormonális változások, menstruáció, stresszhatások és UV-fény.

A HSV-1 vírus a gazdasejtbe való bejutása egy többlépcsős folyamat, ami a virális burok glikoprotein gB és gC heparán szulfátokhoz (HS) való kapcsolódásával kezdődik. A HS a glükózaminoglikánok (GAG) családjába tartozó, heterogén szerkezetű, lineáris poliszacharid, ami általánosan megtalálható az emlős szöveti sejtek felszínén és az extracelluláris mátrixban. A HS- láncok fehérjékhez való kovalens kötődésével proteoglikánok jönnek létre (HSPGs), amelyek sejtfelszíni receptorként/koreceptorként szolgálnak számos ligand számára. Míg a HS-láncoknak bizonyítottan fontos szerepe van a HSV-1 fertőzésben, addig a HSPG-ok magfehérjéinek vírusfertőzésben betöltött szerepéről keveset tudunk. A syndecan család tagjai (syndecan-1, syndecan-2, syndecan-3 és syndecan-4) általánosan megtalálható HSPG-ok az emlős sejtek felszínén, továbbá a syndecan-1 és syndecan-2 fehérjék jelentős mértékben kifejeződnek a HSV-1 fertőzés célsejtjeiként szolgáló epithél sejtek felszínén. Kutatásunk célja az volt, hogy betekintést nyerjünk a syndecan-1 és

syndecan-2 magfehérjék HSV-1 fertőzésben betöltött szerepébe. Fehérje expressziós vizsgálatok segítségével megállapítottuk, hogy HSV-1 fertőzést követően a syndecan-1 és a syndecan-2 fehérjék szintézise megnövekedett mind sejtfelszíni, mind összfehérje szinten, továbbá RNS-interferencia módszerrel kimutattuk, hogy a syndecan-1 és syndecan-2 fehérjék mennyiségének csökkentése negatívan hat a HSV-1 vírusok gazdasejtbe történő bejutására, illetve a vírusfertőzés terjedésére, és elősegíti a fertőzött sejtek túlélését HeLa -sejtekben. Vizsgálataink arra engednek következtetni, hogy a syndecan-1 és syndecan-2 fehérjék mennyiségének változása kapcsolatban áll az aktív vírusfertőzéssel. Összegezve, eredményeink új betekintést nyújtanak a HSPG-ok szerepéről a HSV-1 gazdasejtbe való bejutásának és a vírus sejtről sejtre való terjedésének folyamatába.

Munkánk második részében a kutatócsoportunk által korábban leírt nem kódoló RNS-molekula (PRINS, Psoriasis Susceptibility Related RNA gene Induced by Stress) szerepét vizsgáltuk bakteriális lipopolysacharid kezelést követően, mind immortalizált keratinocita sejtekben (HaCaT), mind elsődleges keratinocyta tenyészetekben.

A pikkelysömör (psoriasis) a populáció 2-4 %-án észlelhető, és világszerte 120-180 millió embert érintő krónikus gyulladásos tünetekkel járó multifaktoriális bőrbetegség. A pikkelysömör pathomechanizmusa még nem ismert pontosan, de a tünetek megjelenésében örökletes genetikai faktorok, környezeti tényezők (a bőr mechanikai, kémiai sérülése, ultraibolya-sugárzás, különféle fertőzések vagy a gyógyszerhasználat) továbbá az immunfolyamatokban bekövetkező abnormalitások is szerepet játszanak. A psoriasis fokozott keratinocita proliferációval, a gyulladást elősegítő sejtek epidermális infiltrációjával jár, ami a bőr felszínén plakkok megjelenéséhez vezet. Noha a számos nagy skálájú génexpressziós vizsgálatoknak köszönhetően tudjuk, hogy a pikkelysömörös epidermiszben több száz gén és fehérje kifejeződése megváltozik, e gének és fehérjék pontos szerepéről a pikkelysömörre való hajlam kialakításában keveset tudunk. A pikkelysömörre hajlamosító örökletes faktorok azonosítása lehetőséget nyújt új diagnosztikus eszközök, valamint preventív terápiás megoldások kidolgozására.

Korábban valós idejű RT-PCR-kísérletek segítségével megállapítottuk, hogy a PRINS-gén eltérő kifejeződési mintázatot mutat a két általunk vizsgált sejtvonalban, egy fehérjeszintézis gátló molekula (cycloheximid), lipopolysaccharid (LPS) kezelést, illetve különböző mikrobiális anyagokkal való kezelést követően. A PRINS gén kifejeződésének

növekedését észleltük a cycloheximid, illetve LPS-kezelést követően HaCaT-sejtekben, ugyanez a primer keratinocitákban nem volt megfigyelhető. Ugyancsak különböző PRINS gén kifejeződési mintázatot tapasztaltunk a két általunk vizsgált sejtvonalban mikrobiális anyagokkal való kezelést követően: míg a PRINS nem-kódoló RNS kifejeződésének markáns növekedését figyeltük meg HaCaT-sejtekben, addig a mikrobiális anyagokkal való kezelés nem váltott ki változást a PRINS kifejeződésében primer keratinocitákban.

Ismert, hogy a mikróbák epitél sejtek általi felismerését a nuclear factor-κB (NF- κB) jelátviteli út aktiválása követi. Annak eldöntésére tehát, hogy a két vizsgált sejtvonalban megfigyelt különbség hátterében a megváltozott NF-κB jelátvitel út áll-e, a PRINS nem-kódoló RNS kifejeződését csökkentettük kis interferáló RNS-ek (siRNS) segítségével HaCaT-sejtekben és a primer keratinocitákban, melyeket előzetesen NF-κB válaszelemet, illetve luciferáz riporter gént tartalmazó konstrukttal transzfektáltunk. Ezután a transzfektált sejtekben nyomon követtük az LPS-kezelés által kiváltott NF-κB aktivációt. Luciferáz riporter esszék segítségével megállapítottuk, hogy a PRINS gén csendesítése nem volt hatással az LPS kiváltotta NF-κB aktivitásra sem HaCaT-sejtekben, sem az elsődleges keratinocitákban. Eredményeink arra engednek következtetni, hogy a PRINS nem-kódoló RNS az NF-κB-től független jelátvivő, vagy a jelátviteli kaszkádban az NF-κB után helyezkedik el. Az LPS-kezelés hatására bekövetkező eltérő PRINS gén aktiváció hátterében más, jelenleg még ismeretlen setjszintű folyamatok állhatnak.

8. REFERENCES

1. Calabrese V, Cornelius C, Dinkova-Kostova AT & Calabrese EJ (2009) Vitagenes, cellular stress response, and acetylcarnitine: relevance to hormesis. *Biofactors* **35**, 146-160.
2. Kultz D (2003) Evolution of the cellular stress proteome: from monophyletic origin to ubiquitous function. *J Exp Biol* **206**, 3119-3124.
3. Bartek J & Lukas J (2001) Pathways governing G1/S transition and their response to DNA damage. *FEBS Lett* **490**, 117-122.
4. Bulavin DV, Amundson SA & Fornace AJ (2002) p38 and Chk1 kinases: different conductors for the G(2)/M checkpoint symphony. *Curr Opin Genet Dev* **12**, 92-97.
5. Brostrom CO & Brostrom MA (1998) Regulation of translational initiation during cellular responses to stress. *Prog Nucleic Acid Res Mol Biol* **58**, 79-125.
6. Feder ME & Hofmann GE (1999) Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. *Annu Rev Physiol* **61**, 243-282.
7. Kato K, Ito H, Iwamoto I, Lida K & Inaguma Y (2001) Protein kinase inhibitors can suppress stress-induced dissociation of Hsp27. *Cell Stress Chaperones* **6**, 16-20.
8. Harkin DP & Hall PA (2000) Measuring a cell's response to stress: the p53 pathway. *Genome Biol* **1**, REVIEWS105.
9. Volcic M, Karl S, Baumann B, Salles D, Daniel P, Fulda S & Wiesmuller L NF-kappaB regulates DNA double-strand break repair in conjunction with BRCA1-CtIP complexes. *Nucleic Acids Res* **40**, 181-195.
10. Garrido C, Brunet M, Didelot C, Zermati Y, Schmitt E & Kroemer G (2006) Heat shock proteins 27 and 70: anti-apoptotic proteins with tumorigenic properties. *Cell Cycle* **5**, 2592-2601.
11. Ashida H, Mimuro H, Ogawa M, Kobayashi T, Sanada T, Kim M & Sasakawa C Cell death and infection: a double-edged sword for host and pathogen survival. *J Cell Biol* **195**, 931-942.
12. Nipic D, Pirc A, Banic B, Suput D & Milisav I Preapoptotic cell stress response of primary hepatocytes. *Hepatology* **51**, 2140-2151.
13. Degterev A, Boyce M & Yuan J (2003) A decade of caspases. *Oncogene* **22**, 8543-8567.
14. Samali A (2007) Apoptosis: a mapped path to cell death. *J Cell Mol Med* **11**, 1212-1213.
15. Shimizu S, Kanaseki T, Mizushima N, Mizuta T, Arakawa-Kobayashi S, Thompson CB & Tsujimoto Y (2004) Role of Bcl-2 family proteins in a non-apoptotic programmed cell death dependent on autophagy genes. *Nat Cell Biol* **6**, 1221-1228.
16. Eskelinen EL (2008) New insights into the mechanisms of macroautophagy in mammalian cells. *Int Rev Cell Mol Biol* **266**, 207-247.
17. Luo S & Rubinsztein DC Apoptosis blocks Beclin 1-dependent autophagosome synthesis: an effect rescued by Bcl-xL. *Cell Death Differ* **17**, 268-277.
18. Sengupta S, Peterson TR & Sabatini DM Regulation of the mTOR complex 1 pathway by nutrients, growth factors, and stress. *Mol Cell* **40**, 310-322.
19. Vanlangenakker N, Vanden Berghe T, Krysko DV, Festjens N & Vandenabeele P (2008) Molecular mechanisms and pathophysiology of necrotic cell death. *Curr Mol Med* **8**, 207-220.

20. Golstein P & Kroemer G (2007) Cell death by necrosis: towards a molecular definition. *Trends Biochem Sci* **32**, 37-43.
21. Hitomi J, Christofferson DE, Ng A, Yao J, Degterev A, Xavier RJ & Yuan J (2008) Identification of a molecular signaling network that regulates a cellular necrotic cell death pathway. *Cell* **135**, 1311-1323.
22. Proskuryakov SY, Konoplyannikov AG & Gabai VL (2003) Necrosis: a specific form of programmed cell death? *Exp Cell Res* **283**, 1-16.
23. Zhu C, Wang X, Xu F, Bahr BA, Shibata M, Uchiyama Y, Hagberg H & Blomgren K (2005) The influence of age on apoptotic and other mechanisms of cell death after cerebral hypoxia-ischemia. *Cell Death Differ* **12**, 162-176.
24. Meylan E & Tschopp J (2005) The RIP kinases: crucial integrators of cellular stress. *Trends Biochem Sci* **30**, 151-159.
25. Bergsbaken T, Fink SL & Cookson BT (2009) Pyroptosis: host cell death and inflammation. *Nat Rev Microbiol* **7**, 99-109.
26. Medzhitov R & Janeway C, Jr. (2000) Innate immunity. *N Engl J Med* **343**, 338-344.
27. Chen GY & Nunez G Sterile inflammation: sensing and reacting to damage. *Nat Rev Immunol* **10**, 826-837.
28. Davis BK, Wen H & Ting JP The inflammasome NLRs in immunity, inflammation, and associated diseases. *Annu Rev Immunol* **29**, 707-735.
29. Thompson AJ & Locarnini SA (2007) Toll-like receptors, RIG-I-like RNA helicases and the antiviral innate immune response. *Immunol Cell Biol* **85**, 435-445.
30. Akira S, Uematsu S & Takeuchi O (2006) Pathogen recognition and innate immunity. *Cell* **124**, 783-801.
31. Brule S, Charnaux N, Sutton A, Ledoux D, Chaigneau T, Saffar L & Gattegno L (2006) The shedding of syndecan-4 and syndecan-1 from HeLa cells and human primary macrophages is accelerated by SDF-1/CXCL12 and mediated by the matrix metalloproteinase-9. *Glycobiology* **16**, 488-501.
32. Kubo T, Wada T, Yamaguchi Y, Shimizu A & Handa H (2006) Knock-down of 25 kDa subunit of cleavage factor Im in Hela cells alters alternative polyadenylation within 3'-UTRs. *Nucleic Acids Res* **34**, 6264-6271.
33. Anastasiadou E, Vaeth S, Cuomo L, Boccellato F, Vincenti S, Cirone M, Presutti C, Junker S, Winberg G, Frati L, Wade PA, Faggioni A & Trivedi P (2009) Epstein-Barr virus infection leads to partial phenotypic reversion of terminally differentiated malignant B cells. *Cancer Lett* **284**, 165-174.
34. Freissler E, Meyer auf der Heyde A, David G, Meyer TF & Dehio C (2000) Syndecan-1 and syndecan-4 can mediate the invasion of OpaHSPG-expressing Neisseria gonorrhoeae into epithelial cells. *Cell Microbiol* **2**, 69-82.
35. Wang SH, Zhang C, Liao CP, Lasbury ME, Durant PJ, Tschang D & Lee CH (2006) Syndecan-1 expression in the lung during Pneumocystis infection. *J Eukaryot Microbiol* **53 Suppl 1**, S122-123.
36. Shukla D & Spear PG (2001) Herpesviruses and heparan sulfate: an intimate relationship in aid of viral entry. *J Clin Invest* **108**, 503-510.
37. Sonkoly E, Bata-Csorgo Z, Pivarcsi A, Polyanka H, Kenderessy-Szabo A, Molnar G, Szentpali K, Bari L, Megyeri K, Mandi Y, Dobozy A, Kemeny L & Szell M (2005) Identification and characterization of a novel, psoriasis susceptibility-related noncoding RNA gene, PRINS. *J Biol Chem* **280**, 24159-24167.

38. Bari L, Bacsa S, Sonkoly E, Bata-Csorgo Z, Kemeny L, Dobozy A & Szell M Comparison of stress-induced PRINS gene expression in normal human keratinocytes and HaCaT cells. *Arch Dermatol Res* **303**, 745-752.
39. Reske A, Pollara G, Krummenacher C, Chain BM & Katz DR (2007) Understanding HSV-1 entry glycoproteins. *Rev Med Virol* **17**, 205-215.
40. Mettenleiter TC (2004) Budding events in herpesvirus morphogenesis. *Virus Res* **106**, 167-180.
41. Whitley RJ & Roizman B (2001) Herpes simplex virus infections. *Lancet* **357**, 1513-1518.
42. Mettenleiter TC (2003) Pathogenesis of neurotropic herpesviruses: role of viral glycoproteins in neuroinvasion and transneuronal spread. *Virus Res* **92**, 197-206.
43. Eisenstein LE, Calio AJ & Cunha BA (2004) Herpes simplex (HSV-1) aseptic meningitis. *Heart Lung* **33**, 196-197.
44. Whitley RJ & Gnann JW (2002) Viral encephalitis: familiar infections and emerging pathogens. *Lancet* **359**, 507-513.
45. Streilein JW, Dana MR & Ksander BR (1997) Immunity causing blindness: five different paths to herpes stromal keratitis. *Immunol Today* **18**, 443-449.
46. Nahmias AJ, Lee FK & Beckman-Nahmias S (1990) Sero-epidemiological and -sociological patterns of herpes simplex virus infection in the world. *Scand J Infect Dis Suppl* **69**, 19-36.
47. Heldwein EE & Krummenacher C (2008) Entry of herpesviruses into mammalian cells. *Cell Mol Life Sci* **65**, 1653-1668.
48. Pertel PE, Fridberg A, Parish ML & Spear PG (2001) Cell fusion induced by herpes simplex virus glycoproteins gB, gD, and gH-gL requires a gD receptor but not necessarily heparan sulfate. *Virology* **279**, 313-324.
49. Spear PG, Eisenberg RJ & Cohen GH (2000) Three classes of cell surface receptors for alphaherpesvirus entry. *Virology* **275**, 1-8.
50. Turner A, Bruun B, Minson T & Browne H (1998) Glycoproteins gB, gD, and gHgL of herpes simplex virus type 1 are necessary and sufficient to mediate membrane fusion in a Cos cell transfection system. *J Virol* **72**, 873-875.
51. Herold BC, WuDunn D, Soltys N & Spear PG (1991) Glycoprotein C of herpes simplex virus type 1 plays a principal role in the adsorption of virus to cells and in infectivity. *J Virol* **65**, 1090-1098.
52. Karasneh GA & Shukla D Herpes simplex virus infects most cell types in vitro: clues to its success. *Virol J* **8**, 481.
53. Herold BC, Visalli RJ, Susmarski N, Brandt CR & Spear PG (1994) Glycoprotein C-independent binding of herpes simplex virus to cells requires cell surface heparan sulphate and glycoprotein B. *J Gen Virol* **75 (Pt 6)**, 1211-1222.
54. Laquerre S, Argnani R, Anderson DB, Zucchini S, Manservigi R & Glorioso JC (1998) Heparan sulfate proteoglycan binding by herpes simplex virus type 1 glycoproteins B and C, which differ in their contributions to virus attachment, penetration, and cell-to-cell spread. *J Virol* **72**, 6119-6130.
55. Clement C, Tiwari V, Scanlan PM, Valyi-Nagy T, Yue BY & Shukla D (2006) A novel role for phagocytosis-like uptake in herpes simplex virus entry. *J Cell Biol* **174**, 1009-1021.

56. Campadelli-Fiume G, Amasio M, Avitabile E, Cerretani A, Forghieri C, Gianni T & Menotti L (2007) The multipartite system that mediates entry of herpes simplex virus into the cell. *Rev Med Virol* **17**, 313-326.
57. Akhtar J & Shukla D (2009) Viral entry mechanisms: cellular and viral mediators of herpes simplex virus entry. *FEBS J* **276**, 7228-7236.
58. Milne RS, Nicola AV, Whitbeck JC, Eisenberg RJ & Cohen GH (2005) Glycoprotein D receptor-dependent, low-pH-independent endocytic entry of herpes simplex virus type 1. *J Virol* **79**, 6655-6663.
59. Nicola AV, McEvoy AM & Straus SE (2003) Roles for endocytosis and low pH in herpes simplex virus entry into HeLa and Chinese hamster ovary cells. *J Virol* **77**, 5324-5332.
60. Tiwari V, Oh MJ, Kovacs M, Shukla SY, Valyi-Nagy T & Shukla D (2008) Role for nectin-1 in herpes simplex virus 1 entry and spread in human retinal pigment epithelial cells. *FEBS J* **275**, 5272-5285.
61. Spear PG, Manoj S, Yoon M, Jogger CR, Zago A & Myscofski D (2006) Different receptors binding to distinct interfaces on herpes simplex virus gD can trigger events leading to cell fusion and viral entry. *Virology* **344**, 17-24.
62. Satoh T, Arii J, Suenaga T, Wang J, Kogure A, Uehori J, Arase N, Shiratori I, Tanaka S, Kawaguchi Y, Spear PG, Lanier LL & Arase H (2008) PILRalpha is a herpes simplex virus-1 entry coreceptor that associates with glycoprotein B. *Cell* **132**, 935-944.
63. Suenaga T, Satoh T, Somboonthum P, Kawaguchi Y, Mori Y & Arase H Myelin-associated glycoprotein mediates membrane fusion and entry of neurotropic herpesviruses. *Proc Natl Acad Sci U S A* **107**, 866-871.
64. Bender FC, Whitbeck JC, Lou H, Cohen GH & Eisenberg RJ (2005) Herpes simplex virus glycoprotein B binds to cell surfaces independently of heparan sulfate and blocks virus entry. *J Virol* **79**, 11588-11597.
65. Arii J, Goto H, Suenaga T, Oyama M, Kozuka-Hata H, Imai T, Minowa A, Akashi H, Arase H, Kawaoka Y & Kawaguchi Y Non-muscle myosin IIA is a functional entry receptor for herpes simplex virus-1. *Nature* **467**, 859-862.
66. Geraghty RJ, Krummenacher C, Cohen GH, Eisenberg RJ & Spear PG (1998) Entry of alphaherpesviruses mediated by poliovirus receptor-related protein 1 and poliovirus receptor. *Science* **280**, 1618-1620.
67. Montgomery RI, Warner MS, Lum BJ & Spear PG (1996) Herpes simplex virus-1 entry into cells mediated by a novel member of the TNF/NGF receptor family. *Cell* **87**, 427-436.
68. Shukla D, Liu J, Blaiklock P, Shworak NW, Bai X, Esko JD, Cohen GH, Eisenberg RJ, Rosenberg RD & Spear PG (1999) A novel role for 3-O-sulfated heparan sulfate in herpes simplex virus 1 entry. *Cell* **99**, 13-22.
69. O'Donnell CD, Kovacs M, Akhtar J, Valyi-Nagy T & Shukla D Expanding the role of 3-O sulfated heparan sulfate in herpes simplex virus type-1 entry. *Virology* **397**, 389-398.
70. Subramanian RP & Geraghty RJ (2007) Herpes simplex virus type 1 mediates fusion through a hemifusion intermediate by sequential activity of glycoproteins D, H, L, and B. *Proc Natl Acad Sci U S A* **104**, 2903-2908.
71. Tiwari V, Clement C, Xu D, Valyi-Nagy T, Yue BY, Liu J & Shukla D (2006) Role for 3-O-sulfated heparan sulfate as the receptor for herpes simplex virus type 1 entry into primary human corneal fibroblasts. *J Virol* **80**, 8970-8980.

72. Tiwari V, Clement C, Scanlan PM, Kowlessur D, Yue BY & Shukla D (2005) A role for herpesvirus entry mediator as the receptor for herpes simplex virus 1 entry into primary human trabecular meshwork cells. *J Virol* **79**, 13173-13179.
73. Esko JD & Lindahl U (2001) Molecular diversity of heparan sulfate. *J Clin Invest* **108**, 169-173.
74. Lindahl U, Kusche-Gullberg M & Kjellen L (1998) Regulated diversity of heparan sulfate. *J Biol Chem* **273**, 24979-24982.
75. O'Donnell CD & Shukla D (2008) The Importance of Heparan Sulfate in Herpesvirus Infection. *Virol Sin* **23**, 383-393.
76. Tumova S, Woods A & Couchman JR (2000) Heparan sulfate proteoglycans on the cell surface: versatile coordinators of cellular functions. *Int J Biochem Cell Biol* **32**, 269-288.
77. Muto T, Miyoshi K, Munesue S, Nakada H, Okayama M, Matsuo T & Noma T (2007) Differential expression of syndecan isoforms during mouse incisor amelogenesis. *J Med Invest* **54**, 331-339.
78. Schofield KP, Gallagher JT & David G (1999) Expression of proteoglycan core proteins in human bone marrow stroma. *Biochem J* **343 Pt 3**, 663-668.
79. Beauvais DM, Burbach BJ & Rapraeger AC (2004) The syndecan-1 ectodomain regulates alphavbeta3 integrin activity in human mammary carcinoma cells. *J Cell Biol* **167**, 171-181.
80. Carey DJ (1997) Syndecans: multifunctional cell-surface co-receptors. *Biochem J* **327 (Pt 1)**, 1-16.
81. Lopes CC, Dietrich CP & Nader HB (2006) Specific structural features of syndecans and heparan sulfate chains are needed for cell signaling. *Braz J Med Biol Res* **39**, 157-167.
82. Deepa SS, Yamada S, Zako M, Goldberger O & Sugahara K (2004) Chondroitin sulfate chains on syndecan-1 and syndecan-4 from normal murine mammary gland epithelial cells are structurally and functionally distinct and cooperate with heparan sulfate chains to bind growth factors. A novel function to control binding of midkine, pleiotrophin, and basic fibroblast growth factor. *J Biol Chem* **279**, 37368-37376.
83. Shworak NW, Shirakawa M, Mulligan RC & Rosenberg RD (1994) Characterization of ryudocan glycosaminoglycan acceptor sites. *J Biol Chem* **269**, 21204-21214.
84. Bobardt MD, Chatterji U, Selvarajah S, Van der Schueren B, David G, Kahn B & Gallay PA (2007) Cell-free human immunodeficiency virus type 1 transcytosis through primary genital epithelial cells. *J Virol* **81**, 395-405.
85. Cheshenko N, Liu W, Satlin LM & Herold BC (2007) Multiple receptor interactions trigger release of membrane and intracellular calcium stores critical for herpes simplex virus entry. *Mol Biol Cell* **18**, 3119-3130.
86. Ethell IM, Irie F, Kalo MS, Couchman JR, Pasquale EB & Yamaguchi Y (2001) EphB/syndecan-2 signaling in dendritic spine morphogenesis. *Neuron* **31**, 1001-1013.
87. Shimabukuro Y, Ichikawa T, Terashima Y, Iwayama T, Oohara H, Kajikawa T, Kobayashi R, Terashima H, Takedachi M, Terakura M, Hashikawa T, Yamada S & Murakami S (2008) Basic fibroblast growth factor regulates expression of heparan sulfate in human periodontal ligament cells. *Matrix Biol* **27**, 232-241.
88. Su G, Blaine SA, Qiao D & Friedl A (2007) Shedding of syndecan-1 by stromal fibroblasts stimulates human breast cancer cell proliferation via FGF2 activation. *J Biol Chem* **282**, 14906-14915.

89. Bos JD, Hulsebosch HJ, Krieg SR, Bakker PM & Cormane RH (1983) Immunocompetent cells in psoriasis. In situ immunophenotyping by monoclonal antibodies. *Arch Dermatol Res* **275**, 181-189.
90. Terui T, Ozawa M & Tagami H (2000) Role of neutrophils in induction of acute inflammation in T-cell-mediated immune dermatosis, psoriasis: a neutrophil-associated inflammation-boosting loop. *Exp Dermatol* **9**, 1-10.
91. Johnson-Huang LM, Lowes MA & Krueger JG Putting together the psoriasis puzzle: an update on developing targeted therapies. *Dis Model Mech* **5**, 423-433.
92. Nestle FO, Kaplan DH & Barker J (2009) Psoriasis. *N Engl J Med* **361**, 496-509.
93. Jiaravuthisan MM, Sasseville D, Vender RB, Murphy F & Muhn CY (2007) Psoriasis of the nail: anatomy, pathology, clinical presentation, and a review of the literature on therapy. *J Am Acad Dermatol* **57**, 1-27.
94. Bowcock AM & Cookson WO (2004) The genetics of psoriasis, psoriatic arthritis and atopic dermatitis. *Hum Mol Genet* **13 Spec No 1**, R43-55.
95. Landells I, MacCallum C & Khraishi M (2008) The role of the dermatologist in identification and treatment of the early stages of psoriatic arthritis. *Skin Therapy Lett* **13**, 4-7.
96. Griffiths CE & Barker JN (2007) Pathogenesis and clinical features of psoriasis. *Lancet* **370**, 263-271.
97. Chamian F, Lowes MA, Lin SL, Lee E, Kikuchi T, Gilleaudeau P, Sullivan-Whalen M, Cardinale I, Khatcherian A, Novitskaya I, Wittkowski KM & Krueger JG (2005) Alefacept reduces infiltrating T cells, activated dendritic cells, and inflammatory genes in psoriasis vulgaris. *Proc Natl Acad Sci U S A* **102**, 2075-2080.
98. Lowes MA, Chamian F, Abello MV, Fuentes-Duculan J, Lin SL, Nussbaum R, Novitskaya I, Carbonaro H, Cardinale I, Kikuchi T, Gilleaudeau P, Sullivan-Whalen M, Wittkowski KM, Papp K, Garovoy M, Dummer W, Steinman RM & Krueger JG (2005) Increase in TNF-alpha and inducible nitric oxide synthase-expressing dendritic cells in psoriasis and reduction with efalizumab (anti-CD11a). *Proc Natl Acad Sci U S A* **102**, 19057-19062.
99. Baker BS, Garioch JJ, Hardman C, Powles A & Fry L (1997) Induction of cutaneous lymphocyte-associated antigen expression by group A streptococcal antigens in psoriasis. *Arch Dermatol Res* **289**, 671-676.
100. Peters BP, Weissman FG & Gill MA (2000) Pathophysiology and treatment of psoriasis. *Am J Health Syst Pharm* **57**, 645-659; quiz 660-641.
101. Barisic-Drusko V & Rucevic I (2004) Trigger factors in childhood psoriasis and vitiligo. *Coll Antropol* **28**, 277-285.
102. Seyhan M, Coskun BK, Saglam H, Ozcan H & Karincaoglu Y (2006) Psoriasis in childhood and adolescence: evaluation of demographic and clinical features. *Pediatr Int* **48**, 525-530.
103. Gudjonsson JE, Thorarinsson AM, Sigurgeirsson B, Kristinsson KG & Valdimarsson H (2003) Streptococcal throat infections and exacerbation of chronic plaque psoriasis: a prospective study. *Br J Dermatol* **149**, 530-534.
104. Horiuchi N, Aiba S, Ozawa H, Sugawara S, Rikiishi H, Kumagai K & Tagami H (1998) Peripheral blood lymphocytes from psoriatic patients are hyporesponsive to beta-streptococcal superantigens. *Br J Dermatol* **138**, 229-235.
105. Langley RG, Krueger GG & Griffiths CE (2005) Psoriasis: epidemiology, clinical features, and quality of life. *Ann Rheum Dis* **64 Suppl 2**, ii18-23; discussion ii24-15.

106. Bernstein CN, Wajda A & Blanchard JF (2005) The clustering of other chronic inflammatory diseases in inflammatory bowel disease: a population-based study. *Gastroenterology* **129**, 827-836.
107. Rehal B, Modjtahedi BS, Morse LS, Schwab IR & Maibach HI Ocular psoriasis. *J Am Acad Dermatol* **65**, 1202-1212.
108. Sommer DM, Jenisch S, Suchan M, Christophers E & Weichenthal M (2006) Increased prevalence of the metabolic syndrome in patients with moderate to severe psoriasis. *Arch Dermatol Res* **298**, 321-328.
109. Gupta MA & Gupta AK (1998) Depression and suicidal ideation in dermatology patients with acne, alopecia areata, atopic dermatitis and psoriasis. *Br J Dermatol* **139**, 846-850.
110. Gupta MA, Gupta AK & Watteel GN (1996) Cigarette smoking in men may be a risk factor for increased severity of psoriasis of the extremities. *Br J Dermatol* **135**, 859-860.
111. Gupta MA, Schork NJ, Gupta AK & Ellis CN (1993) Alcohol intake and treatment responsiveness of psoriasis: a prospective study. *J Am Acad Dermatol* **28**, 730-732.
112. Farkas A & Kemeny L Psoriasis and alcohol: is cutaneous ethanol one of the missing links? *Br J Dermatol* **162**, 711-716.
113. Morris A, Rogers M, Fischer G & Williams K (2001) Childhood psoriasis: a clinical review of 1262 cases. *Pediatr Dermatol* **18**, 188-198.
114. Elder JT, Nair RP & Voorhees JJ (1994) Epidemiology and the genetics of psoriasis. *J Invest Dermatol* **102**, 24S-27S.
115. Farber EM, Bright RD & Nall ML (1968) Psoriasis. A questionnaire survey of 2,144 patients. *Arch Dermatol* **98**, 248-259.
116. Rahman P, Gladman DD, Schentag CT & Petronis A (1999) Excessive paternal transmission in psoriatic arthritis. *Arthritis Rheum* **42**, 1228-1231.
117. Zhang XJ, He PP, Wang ZX, Zhang J, Li YB, Wang HY, Wei SC, Chen SY, Xu SJ, Jin L, Yang S & Huang W (2002) Evidence for a major psoriasis susceptibility locus at 6p21(PSORS1) and a novel candidate region at 4q31 by genome-wide scan in Chinese hans. *J Invest Dermatol* **119**, 1361-1366.
118. Pasic A, Lipozencic J, Ceovic R & Kostovic K (2009) The genetics of psoriasis--selected novelties in 2008. *Acta Dermatovenerol Croat* **17**, 176-181.
119. Alshobaili HA, Shahzad M, Al-Marshood A, Khalil A, Settin A & Barrimah I Genetic background of psoriasis. *Int J Health Sci (Qassim)* **4**, 23-29.
120. Ellinghaus E, Ellinghaus D, Stuart PE, Nair RP, Debrus S, Raelson JV, Belouchi M, Fournier H, Reinhard C, Ding J, Li Y, Tejasvi T, Gudjonsson J, Stoll SW, Voorhees JJ, Lambert S, Weidinger S, Eberlein B, Kunz M, Rahman P, Gladman DD, Gieger C, Wichmann HE, Karlsen TH, Mayr G, Albrecht M, Kabelitz D, Mrowietz U, Abecasis GR, Elder JT, Schreiber S, Weichenthal M & Franke A Genome-wide association study identifies a psoriasis susceptibility locus at TRAF3IP2. *Nat Genet* **42**, 991-995.
121. Capon F, Bijlmakers MJ, Wolf N, Quaranta M, Huffmeier U, Allen M, Timms K, Abkevich V, Gutin A, Smith R, Warren RB, Young HS, Worthington J, Burden AD, Griffiths CE, Hayday A, Nestle FO, Reis A, Lanchbury J, Barker JN & Trembath RC (2008) Identification of ZNF313/RNF114 as a novel psoriasis susceptibility gene. *Hum Mol Genet* **17**, 1938-1945.
122. Cargill M, Schrodi SJ, Chang M, Garcia VE, Brandon R, Callis KP, Matsunami N, Ardlie KG, Civello D, Catanese JJ, Leong DU, Panko JM, McAllister LB, Hansen CB, Papenfuss J, Prescott SM, White TJ, Leppert MF, Krueger GG & Begovich AB (2007) A

large-scale genetic association study confirms IL12B and leads to the identification of IL23R as psoriasis-risk genes. *Am J Hum Genet* **80**, 273-290.
123. Huffmeier U, Uebe S, Ekici AB, Bowes J, Giardina E, Korendowych E, Juneblad K, Apel M, McManus R, Ho P, Bruce IN, Ryan AW, Behrens F, Lascorz J, Bohm B, Traupe H, Lohmann J, Gieger C, Wichmann HE, Herold C, Steffens M, Klareskog L, Wienker TF, Fitzgerald O, Alenius GM, McHugh NJ, Novelli G, Burkhardt H, Barton A & Reis A Common variants at TRAF3IP2 are associated with susceptibility to psoriatic arthritis and psoriasis. *Nat Genet* **42**, 996-999.
124. Liu Y, Helms C, Liao W, Zaba LC, Duan S, Gardner J, Wise C, Miner A, Malloy MJ, Pullinger CR, Kane JP, Saccone S, Worthington J, Bruce I, Kwok PY, Menter A, Krueger J, Barton A, Saccone NL & Bowcock AM (2008) A genome-wide association study of psoriasis and psoriatic arthritis identifies new disease loci. *PLoS Genet* **4**, e1000041.
125. Nair RP, Duffin KC, Helms C, Ding J, Stuart PE, Goldgar D, Gudjonsson JE, Li Y, Tejasvi T, Feng BJ, Ruether A, Schreiber S, Weichenthal M, Gladman D, Rahman P, Schrodi SJ, Prahalad S, Guthery SL, Fischer J, Liao W, Kwok PY, Menter A, Lathrop GM, Wise CA, Begovich AB, Voorhees JJ, Elder JT, Krueger GG, Bowcock AM & Abecasis GR (2009) Genome-wide scan reveals association of psoriasis with IL-23 and NF-kappaB pathways. *Nat Genet* **41**, 199-204.
126. Strange A, Capon F, Spencer CC, Knight J, Weale ME, Allen MH, Barton A, Band G, Bellenguez C, Bergboer JG, Blackwell JM, Bramon E, Bumpstead SJ, Casas JP, Cork MJ, Corvin A, Deloukas P, Dilthey A, Duncanson A, Edkins S, Estivill X, Fitzgerald O, Freeman C, Giardina E, Gray E, Hofer A, Huffmeier U, Hunt SE, Irvine AD, Jankowski J, Kirby B, Langford C, Lascorz J, Leman J, Leslie S, Mallbris L, Markus HS, Mathew CG, McLean WH, McManus R, Mossner R, Moutsianas L, Naluai AT, Nestle FO, Novelli G, Onoufriadis A, Palmer CN, Perricone C, Pirinen M, Plomin R, Potter SC, Pujol RM, Rautanen A, Riveira-Munoz E, Ryan AW, Salmhofer W, Samuelsson L, Sawcer SJ, Schalkwijk J, Smith CH, Stahle M, Su Z, Tazi-Ahnini R, Traupe H, Viswanathan AC, Warren RB, Weger W, Wolk K, Wood N, Worthington J, Young HS, Zeeuwen PL, Hayday A, Burden AD, Griffiths CE, Kere J, Reis A, McVean G, Evans DM, Brown MA, Barker JN, Peltonen L, Donnelly P & Trembath RC A genome-wide association study identifies new psoriasis susceptibility loci and an interaction between HLA-C and ERAP1. *Nat Genet* **42**, 985-990.
127. Stuart PE, Nair RP, Ellinghaus E, Ding J, Tejasvi T, Gudjonsson JE, Li Y, Weidinger S, Eberlein B, Gieger C, Wichmann HE, Kunz M, Ike R, Krueger GG, Bowcock AM, Mrowietz U, Lim HW, Voorhees JJ, Abecasis GR, Weichenthal M, Franke A, Rahman P, Gladman DD & Elder JT Genome-wide association analysis identifies three psoriasis susceptibility loci. *Nat Genet* **42**, 1000-1004.
128. Sun LD, Cheng H, Wang ZX, Zhang AP, Wang PG, Xu JH, Zhu QX, Zhou HS, Ellinghaus E, Zhang FR, Pu XM, Yang XQ, Zhang JZ, Xu AE, Wu RN, Xu LM, Peng L, Helms CA, Ren YQ, Zhang C, Zhang SM, Nair RP, Wang HY, Lin GS, Stuart PE, Fan X, Chen G, Tejasvi T, Li P, Zhu J, Li ZM, Ge HM, Weichenthal M, Ye WZ, Shen SK, Yang BQ, Sun YY, Li SS, Lin Y, Jiang JH, Li CT, Chen RX, Cheng J, Jiang X, Zhang P, Song WM, Tang J, Zhang HQ, Sun L, Cui J, Zhang LJ, Tang B, Huang F, Qin Q, Pei XP, Zhou AM, Shao LM, Liu JL, Zhang FY, Du WD, Franke A, Bowcock AM, Elder JT, Liu JJ, Yang S & Zhang XJ Association analyses identify six new psoriasis susceptibility loci in the Chinese population. *Nat Genet* **42**, 1005-1009.
129. Nair RP, Henseler T, Jenisch S, Stuart P, Bichakjian CK, Lenk W, Westphal E, Guo SW, Christophers E, Voorhees JJ & Elder JT (1997) Evidence for two psoriasis susceptibility

loci (HLA and 17q) and two novel candidate regions (16q and 20p) by genome-wide scan. *Hum Mol Genet* **6**, 1349-1356.
130. Nair RP, Stuart P, Henseler T, Jenisch S, Chia NV, Westphal E, Schork NJ, Kim J, Lim HW, Christophers E, Voorhees JJ & Elder JT (2000) Localization of psoriasis-susceptibility locus PSORS1 to a 60-kb interval telomeric to HLA-C. *Am J Hum Genet* **66**, 1833-1844.
131. Allen MH, Ameen H, Veal C, Evans J, Ramrakha-Jones VS, Marsland AM, Burden AD, Griffiths CE, Trembath RC & Barker JN (2005) The major psoriasis susceptibility locus PSORS1 is not a risk factor for late-onset psoriasis. *J Invest Dermatol* **124**, 103-106.
132. Duffin KC, Chandran V, Gladman DD, Krueger GG, Elder JT & Rahman P (2008) Genetics of psoriasis and psoriatic arthritis: update and future direction. *J Rheumatol* **35**, 1449-1453.
133. Sagoo GS, Cork MJ, Patel R & Tazi-Ahnini R (2004) Genome-wide studies of psoriasis susceptibility loci: a review. *J Dermatol Sci* **35**, 171-179.
134. Bowcock AM, Shannon W, Du F, Duncan J, Cao K, Aftergut K, Catier J, Fernandez-Vina MA & Menter A (2001) Insights into psoriasis and other inflammatory diseases from large-scale gene expression studies. *Hum Mol Genet* **10**, 1793-1805.
135. Nomura I, Gao B, Boguniewicz M, Darst MA, Travers JB & Leung DY (2003) Distinct patterns of gene expression in the skin lesions of atopic dermatitis and psoriasis: a gene microarray analysis. *J Allergy Clin Immunol* **112**, 1195-1202.
136. Zhou X, Krueger JG, Kao MC, Lee E, Du F, Menter A, Wong WH & Bowcock AM (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**, 69-78.
137. Kulski JK, Kenworthy W, Bellgard M, Taplin R, Okamoto K, Oka A, Mabuchi T, Ozawa A, Tamiya G & Inoko H (2005) Gene expression profiling of Japanese psoriatic skin reveals an increased activity in molecular stress and immune response signals. *J Mol Med (Berl)* **83**, 964-975.
138. Quekenborn-Trinquet V, Fogel P, Aldana-Jammayrac O, Ancian P, Demarchez M, Rossio P, Richards HL, Kirby B, Nguyen C, Voegel JJ & Griffiths CE (2005) Gene expression profiles in psoriasis: analysis of impact of body site location and clinical severity. *Br J Dermatol* **152**, 489-504.
139. Gudjonsson JE, Ding J, Li X, Nair RP, Tejasvi T, Qin ZS, Ghosh D, Aphale A, Gumucio DL, Voorhees JJ, Abecasis GR & Elder JT (2009) Global gene expression analysis reveals evidence for decreased lipid biosynthesis and increased innate immunity in uninvolved psoriatic skin. *J Invest Dermatol* **129**, 2795-2804.
140. Romanowska M, al Yacoub N, Seidel H, Donandt S, Gerken H, Phillip S, Haritonova N, Artuc M, Schweiger S, Sterry W & Foerster J (2008) PPARdelta enhances keratinocyte proliferation in psoriasis and induces heparin-binding EGF-like growth factor. *J Invest Dermatol* **128**, 110-124.
141. Elder JT, Fisher GJ, Lindquist PB, Bennett GL, Pittelkow MR, Coffey RJ, Jr., Ellingsworth L, Derynck R & Voorhees JJ (1989) Overexpression of transforming growth factor alpha in psoriatic epidermis. *Science* **243**, 811-814.
142. Nickoloff BJ, Karabin GD, Barker JN, Griffiths CE, Sarma V, Mitra RS, Elder JT, Kunkel SL & Dixit VM (1991) Cellular localization of interleukin-8 and its inducer, tumor necrosis factor-alpha in psoriasis. *Am J Pathol* **138**, 129-140.

143. Detmar M, Brown LF, Claffey KP, Yeo KT, Kocher O, Jackman RW, Berse B & Dvorak HF (1994) Overexpression of vascular permeability factor/vascular endothelial growth factor and its receptors in psoriasis. *J Exp Med* **180**, 1141-1146.
144. Nonomura K, Yamanishi K, Yasuno H, Nara K & Hirose S (1994) Up-regulation of elafin/SKALP gene expression in psoriatic epidermis. *J Invest Dermatol* **103**, 88-91.
145. Van de Kerkhof PC, Gerritsen MJ & de Jong EM (1996) Transition from symptomless to lesional psoriatic skin. *Clin Exp Dermatol* **21**, 325-329.
146. Collins LJ & Penny D (2009) The RNA infrastructure: dark matter of the eukaryotic cell? *Trends Genet* **25**, 120-128.
147. Szell M, Bata-Csorgo Z & Kemeny L (2008) The enigmatic world of mRNA-like ncRNAs: their role in human evolution and in human diseases. *Semin Cancer Biol* **18**, 141-148.
148. Szegedi K, Goblos A, Bacsa S, Antal M, Nemeth IB, Bata-Csorgo Z, Kemeny L, Dobozy A & Szell M Expression and Functional Studies on the Noncoding RNA, PRINS. *Int J Mol Sci* **14**, 205-225.
149. Bell S, Degitz K, Quirling M, Jilg N, Page S & Brand K (2003) Involvement of NF-kappaB signalling in skin physiology and disease. *Cell Signal* **15**, 1-7.
150. Mercurio F, Young DB & Manning AM (2000) Detection and purification of a multiprotein kinase complex from mammalian cells. IKK signalsome. *Methods Mol Biol* **99**, 109-125.
151. Seitz CS, Lin Q, Deng H & Khavari PA (1998) Alterations in NF-kappaB function in transgenic epithelial tissue demonstrate a growth inhibitory role for NF-kappaB. *Proc Natl Acad Sci U S A* **95**, 2307-2312.
152. Van Antwerp DJ, Martin SJ, Kafri T, Green DR & Verma IM (1996) Suppression of TNF-alpha-induced apoptosis by NF-kappaB. *Science* **274**, 787-789.
153. Sur I, Ulvmar M & Toftgard R (2008) The two-faced NF-kappaB in the skin. *Int Rev Immunol* **27**, 205-223.
154. Ghosh S & Karin M (2002) Missing pieces in the NF-kappaB puzzle. *Cell* **109 Suppl**, S81-96.
155. Desai P & Person S (1998) Incorporation of the green fluorescent protein into the herpes simplex virus type 1 capsid. *J Virol* **72**, 7563-7568.
156. Shukla D, Rowe CL, Dong Y, Racaniello VR & Spear PG (1999) The murine homolog (Mph) of human herpesvirus entry protein B (HveB) mediates entry of pseudorabies virus but not herpes simplex virus types 1 and 2. *J Virol* **73**, 4493-4497.
157. Akhtar J, Tiwari V, Oh MJ, Kovacs M, Jani A, Kovacs SK, Valyi-Nagy T & Shukla D (2008) HVEM and nectin-1 are the major mediators of herpes simplex virus 1 (HSV-1) entry into human conjunctival epithelium. *Invest Ophthalmol Vis Sci* **49**, 4026-4035.
158. Burbach BJ, Friedl A, Mundhenke C & Rapraeger AC (2003) Syndecan-1 accumulates in lysosomes of poorly differentiated breast carcinoma cells. *Matrix Biol* **22**, 163-177.
159. Kis K, Bodai L, Polyanka H, Eder K, Pivarcsi A, Duda E, Soos G, Bata-Csorgo Z & Kemeny L (2006) Budesonide, but not tacrolimus, affects the immune functions of normal human keratinocytes. *Int Immunopharmacol* **6**, 358-368.
160. Magalhaes A, Marcos NT, Carvalho AS, David L, Figueiredo C, Bastos J, David G & Reis CA (2009) Helicobacter pylori cag pathogenicity island-positive strains induce syndecan-4 expression in gastric epithelial cells. *FEMS Immunol Med Microbiol* **56**, 223-232.

161. Lewis DA, Hengeltraub SF, Gao FC, Leivant MA & Spandau DF (2006) Aberrant NF-kappaB activity in HaCaT cells alters their response to UVB signaling. *J Invest Dermatol* **126**, 1885-1892.
162. Takizawa H (1998) Airway epithelial cells as regulators of airway inflammation (Review). *Int J Mol Med* **1**, 367-378.
163. Laberge S & El Bassam S (2004) Cytokines, structural cells of the lungs and airway inflammation. *Paediatr Respir Rev* **5 Suppl A**, S41-45.
164. Barker JN, Mitra RS, Griffiths CE, Dixit VM & Nickoloff BJ (1991) Keratinocytes as initiators of inflammation. *Lancet* **337**, 211-214.
165. Trybala E, Liljeqvist JA, Svennerholm B & Bergstrom T (2000) Herpes simplex virus types 1 and 2 differ in their interaction with heparan sulfate. *J Virol* **74**, 9106-9114.
166. Menozzi FD, Reddy VM, Cayet D, Raze D, Debrie AS, Dehouck MP, Cecchelli R & Locht C (2006) Mycobacterium tuberculosis heparin-binding haemagglutinin adhesin (HBHA) triggers receptor-mediated transcytosis without altering the integrity of tight junctions. *Microbes Infect* **8**, 1-9.
167. Barth H, Schafer C, Adah MI, Zhang F, Linhardt RJ, Toyoda H, Kinoshita-Toyoda A, Toida T, Van Kuppevelt TH, Depla E, Von Weizsacker F, Blum HE & Baumert TF (2003) Cellular binding of hepatitis C virus envelope glycoprotein E2 requires cell surface heparan sulfate. *J Biol Chem* **278**, 41003-41012.
168. Giroglou T, Florin L, Schafer F, Streeck RE & Sapp M (2001) Human papillomavirus infection requires cell surface heparan sulfate. *J Virol* **75**, 1565-1570.
169. Shukla SY, Singh YK & Shukla D (2009) Role of nectin-1, HVEM, and PILR-alpha in HSV-2 entry into human retinal pigment epithelial cells. *Invest Ophthalmol Vis Sci* **50**, 2878-2887.
170. Fuki IV, Meyer ME & Williams KJ (2000) Transmembrane and cytoplasmic domains of syndecan mediate a multi-step endocytic pathway involving detergent-insoluble membrane rafts. *Biochem J* **351 Pt 3**, 607-612.
171. Rapraeger A, Jalkanen M, Endo E, Koda J & Bernfield M (1985) The cell surface proteoglycan from mouse mammary epithelial cells bears chondroitin sulfate and heparan sulfate glycosaminoglycans. *J Biol Chem* **260**, 11046-11052.
172. Couchman JR (2003) Syndecans: proteoglycan regulators of cell-surface microdomains? *Nat Rev Mol Cell Biol* **4**, 926-937.
173. Grootjans JJ, Zimmermann P, Reekmans G, Smets A, Degeest G, Durr J & David G (1997) Syntenin, a PDZ protein that binds syndecan cytoplasmic domains. *Proc Natl Acad Sci U S A* **94**, 13683-13688.
174. Chaturvedi V, Qin JZ, Denning MF, Choubey D, Diaz MO & Nickoloff BJ (2001) Abnormal NF-kappaB signaling pathway with enhanced susceptibility to apoptosis in immortalized keratinocytes. *J Dermatol Sci* **26**, 67-78.
175. Hoshino K, Takeuchi O, Kawai T, Sanjo H, Ogawa T, Takeda Y, Takeda K & Akira S (1999) Cutting edge: Toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: evidence for TLR4 as the Lps gene product. *J Immunol* **162**, 3749-3752.
176. Underhill DM, Ozinsky A, Hajjar AM, Stevens A, Wilson CB, Bassetti M & Aderem A (1999) The Toll-like receptor 2 is recruited to macrophage phagosomes and discriminates between pathogens. *Nature* **401**, 811-815.
177. Pivarcsi A, Szell M, Kemeny L, Dobozy A & Bata-Csorgo Z (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**, 206-213.

178. Olaru F & Jensen LE Chemokine expression by human keratinocyte cell lines after activation of Toll-like receptors. *Exp Dermatol* **19**, e314-316.
179. Maas-Szabowski N, Starker A & Fusenig NE (2003) Epidermal tissue regeneration and stromal interaction in HaCaT cells is initiated by TGF-alpha. *J Cell Sci* **116**, 2937-2948.
180. Szegedi K, Sonkoly E, Nagy N, Nemeth IB, Bata-Csorgo Z, Kemeny L, Dobozy A & Szell M The anti-apoptotic protein G1P3 is overexpressed in psoriasis and regulated by the non-coding RNA, PRINS. *Exp Dermatol* **19**, 269-278.
181. Cheriyath V, Leaman DW & Borden EC Emerging roles of FAM14 family members (G1P3/ISG 6-16 and ISG12/IFI27) in innate immunity and cancer. *J Interferon Cytokine Res* **31**, 173-181.
182. Szell M, Bata-Csorgo Z, Koreck A, Pivarcsi A, Polyanka H, Szeg C, Gaal M, Dobozy A & Kemeny L (2004) Proliferating keratinocytes are putative sources of the psoriasis susceptibility-related EDA+ (extra domain A of fibronectin) oncofetal fibronectin. *J Invest Dermatol* **123**, 537-546.
183. Bata-Csorgo Z, Hammerberg C, Voorhees JJ & Cooper KD (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**, 317-327.

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.

Appendix

I.

II.