Mechanism of cytosolic nucleic acid induced IL-23 expression of human keratinocytes

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Summary of the PhD Thesis

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Szeged

2022

List of publications

Scientific papers included in this thesis:

I. **Kelemen E**., Ádám É., Sági S.M., Göblös A., Kemény L., Bata-Csörgő Z., Széll M. and Danis J.: Psoriasis-Associated Inflammatory Conditions Induce IL-23 mRNA Expression in Normal Human Epidermal Keratinocytes, *Int J Mol Sci* 23(1):540 (2022)

IF:5.924

II. **Kelemen E**., Bozó R., Groma G., Bata-Csörgő Z., Kemény L., Danis J., Széll M.: The Psoriatic Non-Lesional skin: A Battlefield between Susceptibility and Protective Factors, *J Invest Dermatol* 141(12):2785-2790 (2021)

IF:8.551

Publications not directly related to the thesis:

III. Danis.J, **Kelemen E**., Rajan N., Nagy N., Széll M., Ádám É: TRAF3 and NBR1 both influence the effect of the disease-causing CYLD(Arg936X) mutation on NF-kB activity, *Exp Dermatol* 30(11):1705-1710 (2021)

IF:3.96

(Independent citation: 0, Self citation:1, Cummulative:1)

IV. **Kelemen E**., Danis J, Göblös A., Bata-Csörgő Z., Széll M: Exosomal long non-coding RNAs as biomarkers in human diseases, *EJIFCC* 30(2):224-236 (2019)

IF:0

(Independent citation: 27, Self citation:0, Cummulative:27)

V. Ámon J., Keisham K., Bokor E., **Kelemen E**., Vágvölgyi C., Hamari Z: Sterigmatocystin production restricted to hyphae located in the proximity of hülle cells, *J Basic Microbiol* 58(7):590-596 (2018)

IF:2.281

(Independent citation: 5, Self citation:0, Cummulative:5)

1. Introduction

1.1. Free nucleic acid induced immune responses in the skin and in psoriasis

The skin is the largest organ of the human body, which provides the first line of defense against environmental, chemical and physical injury and infection by acting as an active participant of the innate immune system. The upper layer of the skin is the epidermis. It's key function is to form a protective interface separating the individual from the external environment.

After physical trauma or infection, keratinocytes release LL-37, which binds DNA and RNA fragments released by damaged skin cells. LL-37 and self-derived nucleic acids form a complex which activates TLR7/9 bearing plasmacytoid dendritic cells, which are normally absent from healthy skin. These cells release proinflammatory cytokines (IL-1β, IL-6 and TNF) and interferons (IFNs) and through these have an important role in the development of psoriasis also by activating keratinocytes and myeloid dendritic cells. The number of myeloid dermal dendritic cells is elevated in psoriatic skin, and the mature cells migrate to skin-draining lymph nodes to present antigen to naive T-cells. T-cells are also critical for the initiation phase of the disease as their interaction with activated dermal dendritic cells is a central event in the development of plaque formation and the resulting creation of an IL-23/IL-17 inflammatory environment. In this environment, IL-23 derived from dendritic cells and macrophages promotes the effector functions of T helper 17 (Th17) and cytotoxic cells. Taken together, these data indicate that cytosolic nucleic acid fragments, which are recognized as PAMPs and DAMPs, are highly abundant in the psoriatic skin and their presence can lead to the chronic activation of professional immune cells. This in turn, leads to the thickening of the epidermis and the formation of inflammed plaques charactheristic for psoriatic skin.

The increased free nucleic acid level might be the consequence of reduced deoxyribonuclease activity in keratinocytes and disturbed ribonuclease activity in psoriatic skin. Increased serum mitochondrial DNA levels originating from mitochondrial dysregulation can act as a DAMP. In psoriatic skin, neutrophils and neutrophil extracellular traps (NETs) might be bound by LL37, and these complexes activate inflammatory reactions that could possibly cause a self-exciting cycle contributing to chronic inflammation in psoriasis [30]. Interferon-γ (IFN-γ), produced by Th1 and Th17 cells can also contribute to the recognition of nucleic acids. The expression of IFN-γ is elevated in psoriatic lesional skin and in the serum of the patients, thereby, this cytokine is able to prime nucleic-acid-induced inflammatory responses in keratinocytes and drive IL-23 expression.

1.2. The role of IL-23 in psoriasis

Many recent publications suggests that the Th17/interleukin-23 axis plays a dominant role in the development of psoriasis, as it promotes chronic inflammation. IL-23 is responsible for the development of Th17 cells, leading to a production of IL-17 and IL-22 cytokines, which are involved in the pathogenesis of psoriasis. Keratinocytes and activated antigen-presenting cells (Langerhans cells, macrophages and dendritic cells) all produce IL-23, the expression of which is elevated in psoriatic lesional skin, leading to an increased number of Th17 cells. Recently, IL-23 targeting therapies for psoriasis have become widely used.

IL-23 is a heterodimeric cytokine consisting of a unique p19 and p40 subunit, the latter is shared with IL-12. Increased levels of these two subunits and the IL-23 receptor were found in psoriatic skin of patients, suggesting that IL-23, rather than IL-12, plays a role in disease pathogenesis. Ustekinumab, a monoclonal antibody against the common p40 subunit is a highly effective treatment for psoriasis, and the antibodies targeting the p19 subunit (guselkumab, tildrakizumab and risankizumab) also show promising impact for symptom amelioration.

1.3. PRINS long non-coding RNA

At the beginning of the 2000s a long non-coding RNA referred to as psoriasis-susceptibility-related RNA gene induced by stress (PRINS) was identified by a differential display experiment in our research group. The 3’ end of the PRINS is 100% similar to the AK022045 RNA sequence. PRINS exhibited higher levels in the uninvolved skin of psoriatic patients than in lesional or healthy epidermis, which suggested its possible contribution to psoriasis susceptibility. Different sets of cellular stressors like ultraviolet-B (UV-B) irradiation, starvation, translation inhibition and hypoxia results modified expression of PRINS. Furthermore, silencing of PRINS in HaCaT cells during stress exposure decreased cellular viability. These results suggested that PRINS has a protective role in cells exposed to stress.

According to our current knowledge we hypothesize that the PRINS evolutionarily young, primate specific long non-coding RNA is differentially expressed in psoriasis, plays a role in keratinocyte stress response and thus in the disease pathogenesis. It has also been shown that PRINS binds directly to the mRNAs of IL-6 and CCL-5 (RANTES) at specific binding sites and eventually destabilizes these mRNAs, leading to a decrease in their accumulation. All these data together indicate that PRINS also has a restrictive role in inflammatory processes.

2. Aims

The aim of our research group is to clarify the details of nucleic acid induced innate immune mechanisms in keratinocytes. In the frame of this project a QPCR array (containing 84 psoriasis relevant genes) was carried out to identify genes showing altered expression as a result of nucleic acid analogue treatment. One of the genes showing the highest change in transcription was the IL-23 cytokine. Therefore we aimed to:

- reveal details of IL-23 expression pathways in keratinocytes by analyzing the free nucleic acid induced signal transduction pathways, and pattern recognition receptors, and study how they contribute to IL-23 expression in human keratinocytes
- examine whether PRINS long non-coding RNA has a regulatory role in nucleic acid induced IL-23 production in human keratinocytes

3. Materials and Methods

- HPV-KER cell line and NHEKs (normal human epidermal keratinocytes) were transfected with 1 μg/ml poly deoxyadenylic acid-poly deoxythymidylic acid double-stranded homopolymer (poly(dA:dT), 0.666 μg/ml polyinosinic-polycytidylic acid (poly(I:C) or with 1 μg PRINS overexpressing plasmid DNA using the X-tremeGene 9 transfection reagent.
- For siRNA-mediated gene silencing of PRRs, the TLR3, RIG-1, IFIH-1, cGAS siRNAs or Non-Targeting Pool constructs were used at a final concentration of 40 nM for transfection with the X-tremeGene siRNA transfection reagent.
- For inhibition experiments, cells were incubated 1 hour prior to poly(dA:dT)/poly(I:C) transfection with specific inhibitors of NF-κB (10 μM), STAT-1 (Fludarabine, 10 μM), STAT-3 (5 μM), JNK (10 μM), MEK-1 (20 μM) and p38 (10 μM).
- At indicated time points after transfection, cells were harvested in TRIzol® Reagent and total RNA was isolated and DNAse treated by Turbo DNA-free Kit. During cDNA synthesis, 1 μg total RNA was reverse transcribed by the EvoScript Universal cDNA master mix.
- Real-time RT-PCR experiments were carried out with the Universal Probe Library or the TaqMan Gene expression system and using a C1000 Touch Thermal Cycler. Relative mRNA levels were calculated using the $\Delta\Delta C_t$ method.
- Using INTARNA, sequence complementarity between PRINS (AK022045) and the mRNA of IL-23 (NM_016584.3) was analyzed. The *in silico* identified putative PRINS binding site sequence of IL-23 cDNA was synthesized and inserted into the pmirGLO vector, resulting the pmirGLO-IL23BS construct. HEK293 cells were transiently transfected by the PRINSpcDNA3.1 or pmirGLO plasmid as controls. Cells were also co-transfected with 0.5 μg pmirGLO-IL23BS or empty pmirGLO plasmid together with the PRINSpcDNA3.1 vector. In each transfection experiment 0.025 μg Renilla-luciferase-expressing pGL4.75 hRluc/CMV plasmid was used as internal control. Cells were harvested and lysated after 24h. Luciferase activities were measured using the Firefly & Renilla Dual Luciferase Assay Kit and SYNERGY/HTX Multi-Mode reader. The luciferase activity derived from the pmirGLO plasmids was normalized to the activity of the Renilla-luciferase activity.
- Experiments were carried out in duplicates with at least three biological repeats. For statistical analysis, a one-tailed, paired Students's t-test was used with correction for multiple comparisons. The significance level was set at $p \leq 0.05$. Pearson's correlation calculation was used to analyze correlation between IL-23 mRNA induction and PRINS overexpression mediated IL-23 mRNA depletion.

4. Results

4.1. Psoriasis-relevant gene expression pattern is modulated by free nucleic acid analogues

To identify genes responding with altered expression in NHEKs to treatment with the synthetic nucleic acid analogues poly(I:C) and poly(dA:dT), a qPCR array containing 84 psoriasis-relevant genes was performed. In this experiment, we observed that 14 genes were not expressed by the cells, whereas the expression of 15 additional genes, mostly antimicrobial peptides, was not altered after treatment with either poly(I:C) or poly(dA:dT). We identified 37 genes that were induced by both treatments. Increased expression in response to only poly(I:C) treatment was observed for 7 additional genes.

Most importantly, we observed that in a manner similar to other inflammatory cytokines, such as IL-6 and TNF-α, IL-23 expression increased in response to both poly(dA:dT) and poly(I:C) treatment. Since IL-23 is known to play an important role in psoriasis pathogenesis, we designed further experiments to examine the molecular mechanism of free nucleic acid induced IL-23 transcription in human keratinocytes.

In the qPCR array, mRNA samples derived from four healthy donors were used, and the first validation experiments were carried out on the same mRNA set. These results confirmed that poly(I:C) induced significant increase in IL-23 mRNA, whereas poly(dA:dT) induced an upward trend in expression. To expand the validation of the array, we included additional independent donors (n=6) and observed significantly higher IL-23 expression after both treatments, however, the treatment with poly(I:C) had a more pronounced effect in this experiment as well.

4.2. Poly(I:C) and poly(dA:dT) induce IL-23 mRNA expression with different kinetics

Previously, we identified kinetic differences in poly(I:C)- and poly(dA:dT)-induced inflammatory responses in keratinocytes. To clarify this in the case of IL-23, we measured IL-23 mRNA levels at different time points after nucleic acid treatments. The peak expression of IL-23 was observed later, at 24 hours after poly(I:C) transfection, while a slowly rising tendency was detected after poly(dA:dT) treatment, and these levels were lower than those observed after poly(I:C) treatment. To further confirm our results, HPV-KER cells were also included in our experiments. In the HPV-KER cells similarly what we detected in NHEKs we also observed a peak expression of IL-23 mRNA after 24 hours of poly(I:C) treatment, but after poly(dA:dT) transfection, we detected a rising tendency.

4.3. Psoriasis-specific stimuli induce IL-23 mRNA expression

To compare the effects of psoriasis specific-cytokines with the effects of nucleic acids on IL-23 mRNA expression in keratinocytes, we treated the cells with IL-17A, IL-12, TNF-α and IL-23, and synthetic nucleic acid analogues imiquimod (IMQ), poly(dA:dT) and poly(I:C. The highest IL-23 expression was induced by poly(I:C), whereas poly(dA:dT) and TNF-α treatment also elevated IL-23 transcription but to a lesser extent. Other psoriasis-relevant cytokines and IMQ did not affect IL-23 mRNA expression in keratinocytes.

These results indicate that synthetic nucleic acids, modelling free cellular nucleic acids—especially poly(I:C)—play an important role in the psoriasis-associated inflammatory processes by inducing elevated IL-23 levels in human keratinocytes.

4.4. TLR3 is the main nucleic acid sensing receptor conveying IL-23 mRNA expression in NHEKs

In psoriasis, nucleic acid fragments originating from the tissue or pathogens are present and recognized as danger signals for disease development. Several pathogen recognition receptors (PRRs) that recognize these RNA and DNA fragments and induce inflammatory mechanisms, and many of them are expressed both in keratinocytes and professional immune cells. We aimed to examine which of these receptors plays an important role in mediating nucleic acid induced IL-23 mRNA expression in keratinocytes. To this end, siRNA-mediated silencing was carried out to decrease the levels of TLR-3, RIG-I, IFIH1(MDA-5) and cyclic GMP-AMP synthase (cGAS). The effect of silencing was verified by qPCR. Our results showed that poly(I:C)-induced IL-23 mRNA expression is mediated primarily by TLR3 in NHEKs. Silencing none of the receptors in our experiments did affect the influence of poly(dA:dT) on IL-23 transcription. The same experiments carried out in HPV-KER cells revealed that besides TLR3, several other PRRs like RIG-1, IFIH, cGAS might also regulate poly(I:C) and poly(dA:dT) induced IL-23 mRNA expression in these cells.

4.5. Multiple pathways transmit free nucleic acid signals to mediate IL-23 mRNA expression

In our studies, we examined six pathways that were previously shown to be affected by nucleic acids in other cell types. The activity of a major component of each of these pathways was decreased by applying specific inhibitors, such as Bay 11-7085 for NF-κB, PD95089 for dual specificity MEK1/2, SB203580 for p38, SP600125 for JNK, fludarabine for STAT-1 and Stattic for STAT-3. Keratinocytes were preincubated with the inhibitor for an hour before transfection with poly(I:C) or poly(dA:dT). Our results showed that poly(I:C)-induced IL-23

mRNA expression is mediated by JNK, ERK1/2, NF-κB and STAT3 pathways, downstream of TLR3 receptor. However, inhibition of either of these pathways had no effect on poly(dA:dT)-induced IL-23 mRNA expression. Furthermore, we also observed that similarly to NHEKs poly(I:C) induced IL-23 mRNA expression is mediated by, ERK1/2 and NF- κB pathways in HPV-KER cells.

4.6.The effect of the PRINS long non-coding RNA shows a great inter-individual difference on nucleic acid analogue induced IL-23 mRNA expression in human keratinocytes

To identify additional targets for PRINS-mediated regulation in NHEKs, a qPCR-array was used to identify genes showing altered expression as a consequence of nucleic acid challenge and PRINS overexpression. A PRINS overexpressing construct was transiently transfected into NHEK cells, and the cells were subsequently treated with poly(I:C) or poly(dA:dT). In the qPCR-array impaired upregulation of inflammatory genes including cytokines, chemokines, receptors and effector molecules was observed. We have previously shown that PRINS binds to the mRNA of IL-6 and CCL-5 leading to their degradation. These mRNAs were also downregulated in the qPCR-array, validating our results. The qPCR array identified another psoriasis-related transcript, IL-23 mRNA, that was downregulated in the PRINS overexpressing keratinocytes. We confirmed this result by validating the array data with keratinocytes from the same donors as used for the qPCR array.

To further support the role of PRINS in IL-23 mRNA regulation, we examined the effect of the silencing or overexpression of PRINS on the nucleic acid induced IL-23 mRNA expression in NHEK cells derived from additional individuals. These experiments confirmed that PRINS overexpression significantly reduced IL-23 mRNA expression after poly(dA:dT) treatment, whereas a decreasing trend was observed after poly(I:C) treatment. We observed an opposite tendency when PRINS expression was decreased by silencing. However, statistical significance was not reached in many cases, since large inter-individual differences among the samples derived from independent donors can be observed.

Bioinformatic analysis of IL-6 and CCL-5 predicted an mRNA-lncRNA binding site and our functional studies confirmed the physical link betwwen these molecules. Thus we attempted to determine whether a PRINS-interaction site is present in IL-23 mRNA. The predicted binding site in PRINS to the IL-23 mRNA partly overlaps the previously identified IL-6 mRNA binding sequence. Since IL-23 is an extremely polymorphic gene, we hypothesized that a reason for the large inter-individual differences observed between the donors might be due to single-

nucleotide variants (SNPs) at the putative PRINS interaction site in IL-23, which could interfere with PRINS binding. Moreover, certain SNPs of the IL-23 gene are linked to psoriasis susceptibility. Searching the NCBI database for polymorphisms at the putative binding site, 13 SNPs were identified on the IL-23 gene. We sequenced the putative binding site of the IL-23 gene from each donor and compared the sequences. Sequence variants of the gene could not be associated with the IL-23 mRNA expression response to PRINS overexpression. To confirm the putative binding between PRINS and the IL-23 mRNA identified *in silico*, we used a luciferase-based vector carrying cloning sites at the 3' end of the luciferase gene. The putative PRINS interacting sequence of the IL-23 cDNA was inserted into this vector (pmirGLO-IL23BS). In the absence of any binding partner, transfected cells exhibit luciferase activity. If a binding event were to occur between PRINS and the IL-23 mRNA sequence, the luciferase mRNA would be destabilized, and luciferase activity would not be detected. Upon co-transfection the cells with pmirGLO-IL23BS- and PRINS-expressing plasmids, we observed hardly any difference in luciferase activity in the presence or absence of the interaction site, indicating no physical interaction through this sequence between IL-23 mRNA and PRINS.

However, we noticed that IL-23 mRNA downregulation by PRINS overexpression was more apparent in those donor samples where initial induction of IL-23 mRNA transcription by nucleic acids was the highest, suggesting a correlation. Pearson's correlation analysis confirmed a significant, moderate negative correlation between the extent of IL-23 mRNA expression by nucleic acid induction and the rate of decrease upon PRINS overexpression. This suggest that an adequately high level of IL-23 mRNA is required for the achievement of regulatory effect of PRINS on IL-23 mRNA expression.

5. Discussion

Nucleic acid fragments are important PAMPs or DAMPs that induce innate immune processes of professional and non-professional immune cells. The accumulation of RNA and DNA fragments in keratinocytes has pathogenic role in the psoriatic parakeratosis. Increased expression of the subunits of IL-23 and its receptor were found in psoriatic lesional skin, which suggest that this cytokine play a role in psoriatic inflammation. However, there are no available data about the possible connection between the effect of these inflammatory agents.

In this study we aimed to investigate the mechanism of how nucleic acid challenge regulates IL-23 mRNA expression in keratinocytes and in the HPV-KER cell line. In our previous experiments we found that NHEK cells and the HPV-KER cell line exhibited similar cytokine mRNA expression, but high intra-individual differences were observed in the inflammatory inductions of NHEKs. Our results showed that both poly(I:C) and poly(dA:dT) induce IL-23 mRNA expression in keratinocytes, but to a different degree and with different kinetics. The difference might originate from differences in recognition of RNA and DNA by PRRs. Specific PRRs that are capable of sensing nucleic acids in keratinocytes have already been wildly described. These receptors recognize RNA and DNA fragments from both native and pathogenic origin and induce inflammatory mechanisms. TLR3 recognizes dsRNAs from viruses as well as poly(I:C), and it is essential for the production of the IL-12p40 subunit. TLR3 was also shown to induce IL-23p19 expression through IRF6. RIG-I and IFIH1 belong to the RIG-like receptors. RIG-I predominantly recognizes short dsRNA, while IFIH1 senses long dsRNA from viruses. However, it was previously shown that poly(dA:dT) is transcribed into dsRNA by RNA polymerase III before recognized by the RIG-I receptor, which, as suggested previously, might explain the kinetic differences between poly(I:C) and poly(dA:dT) treatments. Recently, cGAS was described as the major PRR to recognize cytosolic DNA fragments. To date, the involvement of these receptors in the production of IL-23 by keratinocytes has not been addressed. Our results demonstrated that, whereas poly(I:C)-mediated IL-23 mRNA expression was decreased by silencing of TLR3 in both NHEKs and HPV-KER cells, in line with previous reports, silencing of none of the studied receptors had an effect on poly(dA:dT)-induced IL-23 mRNA expression in NHEKs. Nevertheless, we emphasize that the level of induction after poly(dA:dT)-treatment never approached the level induced by poly(I:C) treatment, which might explain the negative results in the silencing experiments. However in HPV-KER cells we observed higher IL-23 expression levels upon poly(dA:dT) treatment. Our experiments were carried out on NHEK cells isolated from four

healthy donors and even in this small data set, we observed differences in the reactions of the individuals. These results drew our attention to the importance of differential sensitivity of individuals in responses to different agents. We could also observe differences between NHEKs and HPV-KER cells: in NHEKs only TLR3 seems to participate in nucleic acid induced IL-23 production, while in HPV-KER cells silencing several receptors caused a decrease in the level of nucleic acid induced IL-23 transcription.

The nuclear-factor (NF)-κB signaling pathway affects cell survival, proliferation and anti-apoptotic effects of lymphocytes and keratinocytes and it is known that TNF-α induces Th17 to produce pro-inflammatory cytokines through this pathway in psoriatic lesions. In eukaryotic cells, three mitogen-activated protein kinase (MAPK) cascades have been identified: ERK, JNK and p38. The ERK signaling pathway plays an important role in cell proliferation and differentiation, while JNK and p38 are mainly related to the stress response and apoptosis of cells. The JAK/STAT signaling pathway, also known as the IL-6 signaling pathway, is involved in many biological processes, such as cell proliferation, differentiation and apoptosis and is also closely related to many immune and inflammatory diseases. It has been shown that, in human keratinocytes, poly(I:C) induces NF-κB, p38 and STAT-1 signaling, whereas poly(dA:dT) treatment activated NF-κB, p38 and JNK signaling in human melanocytes.

According to our results inhibition of the ERK-1, JNK, NF-κB and STAT3 pathways resulted in a decrease in poly(I:C)-induced IL-23 mRNA expression of keratinocytes, suggesting that all of these pathway components play a role in the RNA-induced production of IL-23 mRNA under inflammatory circumstances. Blockage of these pathways did not result in complete decay of IL-23 expression, showing the involvement of other, related pathways including the known role of TLR3 signaling through IRF6. These suggest that poly(I:C) induced IL-23 mRNA expression in NHEKs is mainly mediated by TLR3 driven activation of several parallel pathways. Interestingly, similarly to silencing of the major nucleic acid sensing PRRs, inhibition of these major pathways did not have an effect on poly(dA:dT)-induced IL-23 mRNA expression, suggesting the importance of alternative receptors and pathways in DNA-induced IL-23 mRNA expression that have not yet been identified. The involvement of other, less exposed receptors or pathways in poly(dA:dT)-induced IL-23 mRNA expression would also explain the observed lower expression compared to poly(I:C)-induced expression levels.

PRINS long non-coding RNA has been characterized as a contributor to the anti-inflammatory state of the epidermis by binding directly to inflammatory molecules, specifically to IL-6 and CCL-5 mRNAs. To identify additional target transcripts of PRINS under inflammatory conditions, nucleic acid induction was applied during PRINS overexpression.

PRINS overexpression led to a decrease in nucleic acid induced IL-23 mRNA expression, and a putative lncRNA-mRNA binding site was identified *in silico*. Although initially we confirmed the decrease in IL-23 mRNA expression upon PRINS overexpression, increasing the number of independent samples lead us to conclude that there were large inter-individual differences among the donors. Since a direct lncRNA–mRNA interaction could be affected by SNPs, their presence was assessed; however, sequencing the putative binding site on the IL-23 gene of the different donors revealed no correlation with the presence of sequence variants and the IL-23 mRNA response to PRINS overexpression. Interaction could also not be confirmed with an experimental approach to evaluate the direct interaction between the IL-23 mRNA putative binding site and the PRINS lncRNA molecule using a luciferase-based binding assay. In further studies, the responses of the donors seemed to be related to the level of the nucleic acid induced IL-23 mRNA expression. A correlation analysis was carried out and confirmed the possible negative correlation between the nucleic acid induced IL-23 mRNA expression levels and the decrease upon PRINS overexpression, suggesting the need for sufficiently high levels of IL-23 mRNA for PRINS regulation to be detected. However, even in cases when induction of IL-23 mRNA expression was sufficiently high, PRINS might act indirectly, rather than by direct interaction with the IL-23 mRNA.

Although IL-23 in psoriasis is predominantly thought to be released by professional immune cells, our results indicated that this cytokine might originate from keratinocytes as well. Nucleic acids activate several PRRs in these cells, leading to the upregulation of several inflammatory molecules, including IL-23. Thus, keratinocyte-derived nucleic acids in the skin might also contribute to the development of psoriasis by elevating the IL-23 levels in keratinocytes through an autocrine mode. Taken together, our data suggest that individual differences in sensitivity towards the levels of nucleic acids in the tissue might lead to different IL-23 levels. These differences might in turn be related to the different reactions towards biological agents used in psoriasis treatment. Detailed analysis of these processes will help us to identify new biomarkers for the development of personalized treatments for the disease.

It has long been debated by the scientific community, whether altered immune cells play an exlusive major role in the development of psoriasis, with keratinocytes acting as a subordinate passive contributors. However, several publications and our own results support the idea that keratinocytes are also active participants in these processes, as they not only play a role in the innate immune response, but also modulate the course of the acquired immune response.

6. Summary, novel findings of the experimental work

In this study we characterized the dsRNA and dsDNA induced IL-23 expression in human epidermal keratinocytes and HPV-KER cells by studying the transcriptional regulation of this cytokine.

- We compared the dsRNA and dsDNA induced IL-23 expression in human epidermal keratinocytes. We found that transient transfection by the synthetic dsRNA and dsDNA analogue poly(I:C) and poly(dA:dT) induced IL-23 transcription in keratinocytes, but to different degree and with different kinetics.
- Silencing of the pattern recognition receptors showed that TLR3 is the main receptor mediating the poly(I:C) specific regulation of IL-23 mRNA expression levels in both NHEKs and HPV-KER cells, however in HPV-KERs other receptors also contribute to this process.
- We showed that the inhibition of the ERK-1, JNK, NF-kB and STAT3 pathways resulted in a decrease in the nucleic acid induced induction of IL-23 mRNA expression in keratinocytes.
- Our results indicate that free nucleic acids contribute to the development of psoriasis by elevating the IL-23 levels through specific receptors and signaling pathways in keratinocytes.
- According to our qPRC array nuclecic acid induced IL-23 expression can be changed by elevated PRINS levels. We confirmed this results by increasing the number of donors and by increasing or decreasing the levels of PRINS in keratinocytes.
- Since we observed very big differences in the samples derived from different individuals, we sequenced the putative bindig site of the donors and found no correlation with the appearance of the variants and the IL-23 response to PRINS overexpression. We also could not detect interaction between the IL-23 putative binding site and the PRINS lncRNA molecule in our luciferase based assay.

Taken together, our data suggest that individual differences in sensitivity towards the levels of nucleic acids in the tissue might lead to different IL-23 levels. These differences might in turn be related to the different reactions towards biological agents used in psoriasis treatment. Detailed analysis of these processes will help us to identify new biomarkers for the development of personalized treatments for the disease.

7. Acknowledgement

I would like to express my deepest gratitude to my supervisors, Prof. Dr. Márta Széll and Dr. Judit Danis, for their constant support and supervision of my work. They always stood by, when I needed their guidance and support, but also provided me enough independence during my scientific work.

I would like to thank Prof. Dr. Lajos Kemény for the opportunity to perform my studies here.

It also gives me a pleasure to thank Dr. Éva Ádám for her ideas and comments related to my work and during the preparation of the manuscripts.

I would like to thank Hedvig Koósné Majzik for the excellent technical assistance.

I am grateful to my collagues Dr. Anikó Göblös, Dr. Beáta Szilvia Bolla, Dr. Lilla Erdei and Dr. Renáta Bozó for introducing and helping me with the research techniques.

During my work at the research group, I was lucky to have been able to participate in the supervision of Stella Márta Sági from the University of Szeged. She has contributed to the manual work which led to the results of this Thesis.

My special thank go to my friends and family, for their constant support, encouragement and endless love, especially to my fiancé who is the most patient man in this world.

This study was supported by several research grants from the National Research, Development and Innovation Office (OTKA K128736 and OTKA FK134355) and by the EU Horizon 2020 Research and Innovation Program under agreement no. 739593.