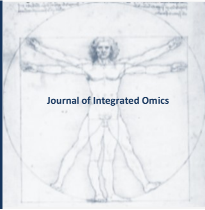

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The potential benefits of shRNA-mediated MMP1 silencing for psoriasis

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Abstract

Matrix metalloproteinases (MMPs) orchestrate structural remodeling of psoriatic skin and accelerate the development of the inflammatory response.

In this paper, we explore whether knocking MMP1 down in epidermal keratinocytes can be beneficial for psoriasis.

We discovered that MMP1 silencing with specific shRNA reduced the migration of epidermal keratinocytes and made the cells susceptible to apoptosis in the presence of interferon-γ. Furthermore, MMP1-deficiency partially normalized the expression of genes involved in the pathogenesis of psoriasis (MMP9, -12, CCNA2, CCND1 and KRT17) and the terminal differentiation (KRT1, -10, IVL and LOR).

In conclusion, MMP1 silencing could be beneficial for psoriasis. MMP1-deficient cells exhibit lower proliferation rate. Moreover, MMP1-silencing makes the cells susceptable to the proinflammatory cytokine IFN-γ, which is abundant in lesional skin. In addition, knocking MMP1 down shifts the balance between proliferation and differentiation toward differentiation. The latter is important for psoriasis, which is a hyperproliferative skin disorder.

Аннотация

Матричные металлопротеиназы (ММР) принимают активное участие в структурной реорганизации эпидермиса, а также в стабилизации воспалительного процесса, которые происходят в пораженной псориазом коже.

Целью данной работы было оценить каким образом РНК-интерференция ММР1 в эпидермальных кератиноцитах человека может повлиять на патогенез псориаза.

Согласно полученным результатам, РНК-интерференция ММР1 в эпидермальных кератиноцитах человека приводит к снижению скоростей миграции и пролиферации данного типа клеток, а добавление в культуральную среду интерферона-γ инициирует их апоптоз. Кроме того, РНК-интерференция ММР1 частично нормализует экспрессию важных для патогенеза болезни генов (MMP9, -12, CCNA2, CCND1 и KRT17), а также генов-маркеров терминальной дифференцировки эпидермальных кератиноцитов (KRT1, -10, IVL и LOR).

Таким образом, РНК-интерференция ММР1 может иметь клиническое значение при псориазе, прежде всего, благодаря выраженному антипролиферативному эффекту. Более того, «нокдаун» ММР1 делает клетки восприимчивыми к провоспалительному цитокину IFN-γ, уровень которого повышен в пораженной болезнью коже. Наконец, РНК-интерференция ММР1 приводит к смещению баланса между пролиферацией и дифференцировкой клеток в сторону их дифференцировки. Последнее особенно важно для псориаза, который является гиперпролиферативным заболеванием кожи.

Keywords: Matrix metalloproteinase 1; Gene silencing; Th1-cytokine; Inflammatory response; Psoriasis.

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1. Introduction

Psoriasis is a chronic inflammatory disease driven by activated T-cells [1]. According to World Health Organization, more than 2% of the entire human population is suffering from psoriasis [2]. In Russia, where about 2.8 million people are diagnosed with psoriasis, the prevalence of the disease is ~1.9%, [3]. The hallmark of psoriasis is the appearance of red flaky-crusty patches covered with silvery seals. The cause of psoriasis is unknown. The skin accumulates immune cells that secrete proinflammatory cytokines. Three of them tumor necrosis factor (TNF), interleukin 17 (IL17) and interferon γ (IFN-γ) are the most important for the pathogenesis of the disease. Their accumulation in the skin leads to activation of epidermal keratinocytes, structural remodeling of the epidermis and development of the plaques. It also results in a faster turnover of epidermal keratinocytes and causes their hyperproliferation. [4].

Small interfering RNAs (siRNAs) that destroy protein-encoding mRNAs are present in any viable cell [5]. Targeting mRNAs, siRNAs prevent their translation into proteins by the ribosomes. Respectively, even partial degradation of mRNA by siRNA decreases protein expression. For this reason, the artificially designed siRNAs, known as small hairpin RNAs (shRNAs), are often used in routine experimental practice to knock down disease-associated genes. For instance, shRNA directed to MMP1 could be used to reduce MMP1 expression in cultured mammalian cells.

Matrix metalloproteinases (MMPs) are a group of zinc-containing, calcium-dependent endopeptidases. In psoriasis, MMPs modify the intercellular contacts by degrading the proteins of hemidesmosomes [6] and desmosomes [7-9]. They also change the composition of the extracellular matrix [10], facilitate the penetration of dermal microcapillaries by the immune cells and influence the biological activity of some cytokines [11].

In the lab, our research is focused on matrix metalloproteinases, such as MMP1, and their role in psoriasis. Previously, we have shown changes in MMP1, -9 and -12 expression in lesional psoriatic skin that coincided with exacerbation of the disease and correlated with the disease severity [12]. The aim of this study was to explore whether MMP1 silencing in epidermal keratinocytes could be beneficial for psoriasis.

2. Material and Methods

2.1 Cell lines and cell culturing:

The experiments were performed on human epidermal keratinocytes HaCaT-MMP1 and HaCaT-KTR that expressed MMP1-specific and scrambled (control) shRNA, respectively. The details on selection, design and cloning of the mentioned shRNAs were described earlier [13,14]. The target sequences used to design specific and scrambled shRNA were CAACAATTTCAGAGAGTAC and GTAAAGGGAACCAACTAACAGA, respectively. The specificity of the selected shRNAs to all know protein-encoding mRNA was verified by "Blastn". The cells were obtained by the method lentiviral tranduction in accordance with the previously described protocol [15].

2.2 Purification of total RNA

Total RNA was extracted with TRIzol reagent (ThermoFisher Scientific, USA) as described earlier [16]. Quality of the obtained RNA samples was verified using non -denaturing 1.5 % agarose gel electrophoresis. The RNA concentration was measured using the fluorimetric Qubit RNA BR Assay Kit (ThermoFisher Scientific) according to the manufacturer's protocol.

2.3 qPCR:

Before the experiment, total RNA was converted to cDNA using MMLV RT kit (Evrogen, Russia) according to the manufacturer's protocol. The real-time PCR experiments were carried out in the Eco real-time PCR system (Illumina, USA). The primers used in this study were taken from the database NCBI Probe [17]. The results were analyzed using the software supplied by Illumina. Each probe was run in triplicates. After all, three independent experiments were performed. The ACTB assay was used as an endogenous control.

2.4 Western blot

Before the experiment, whole cell lysates were obtained as described earlier [18] and protein concentration was measured by Bradford assay (Bio-Rad Laboratories; Richmond, CA, USA). Then, equal amounts of protein (10 μg) were added to Laemmli sample buffer (5% SDS, 25% glycerol, 125 mM TRIS, 0.004% bromphenol blue, 10% β-mercaptoethanol, pH 8.2) and subjected to electrophoresis in 12% polyacrylamide gel. Following transfer to PVDF membrane, samples were probed with primary antibodies (Abcam, USA, Cat #ab137332). Signals were captured using X-Omat K film (Eastman Kodak Co., USA). The films were scanned as image files, and the optical densities of the bands in these image files were quantified using the ImageJ software [19].

2.5 Proliferation assay

The cells were seeded in 6-well plates, 40,000 cells per well. At the indicated time points, randomly chosen samples were treated with 0.25% of trypsin-EDTA solution (PanEco). Then, cells were resuspended, stained with trypan blue (0.2%) and counted in hemocytometer. The obtained values were used for plotting the cell growth curves in linear

coordinates. After all, three independent experiments were performed.

2.6 Scratch assay

Scratch assay was used to assess cell migration rates. The cells were cultured until in 6-well plates until they covered the entire growth surface. Before the experiment, the cell monolayer was scratched with a pipette tip to obtain a 1.2–1.3 mm-wide cell-free area across the center of the well. The remained cells were washed with PBS and cultured for 5–6 days. The representative parts of cell-free areas were photographed daily and quantified with ImageJ software [19].

2.7 Statistics

Data were represented as means±SE. The statistical differences between the means were analyzed by one-way ANOVA. If *p*-values were less than 0.05, means were considered to be significantly different.

3. Results

3.1. Quantitative analysis of MMP1 expression

Evaluation of cDNA (Figure 1A) and protein fractions (Figure 1B) isolated from HaCaT-MMP1 and HaCaT-KTR cells discovered that the mentioned cell lines exhibited different expression levels of MMP1 mRNA and protein. In this respect, the expression levels of MMP1 mRNA and fully -processed catalytically active enzyme in HaCaT-MMP1 did not exceed 15.9 and 16.1% of the corresponding values in HaCaT-KTR cells. In cell homogenates, MMP1 protein was represented by two bands that were identified as proenzyme and catalytically active MMP1 (Mw ~63 and 52 kDa, respectively).

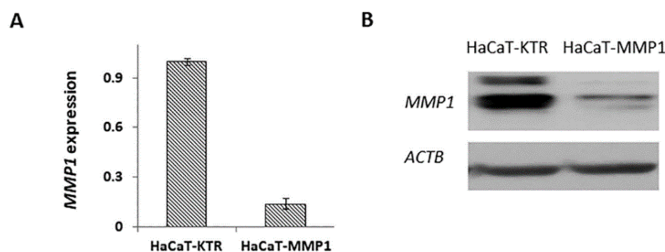

Figure 1 | MMP1 silencing in HaCaT cells. Analysis of gene (A) and protein expression (B) in the cells expressing shRNA directed to MMP1 (HaCaT-MMP1) compared to the cells expressing scrambled shRNA. (HaCaT-KTR). MMP1 expression level in non-stimulated HaCaT cells was referred to as 1. РНК-интерференция MMP1 в эпидермальных кератиноцитах HaCaT. Изменения концентрации мРНК (A) и белка (B) в клетках, экспрессирующих shРНК, специфичную к MMP1 (HaCaT-MMP1) и контрольную, т.н. “scrambled” shRNA (HaCaT-KTR). Соответствующий уровень MMP1 в нетрансдуцированных клетках принимали равным единице

3.2. Changes in cell proliferation

A comparative analysis of cell growth demonstrated that non-stimulated cells as well as the cells stimulated with either TNF or IL17 remained in the active growth phase (Figure 2A-C). The time-dependences of cell growth in linear coordinates suggested that the cells grew monotonously, i.e. the growth curves did not reach saturation for the time of observation. Despite untreated HaCaT-KTR and HaCaT-MMP1 cells did not exhibit significant differences in cell proliferation rate (Figure 2A), the proliferation rates of HaCaT-MMP1 cells treated with the named cytokines were significantly lower compared to HaCaT-KTR cells (Figure 2B and C). In addition, an exposure of HaCaT-MMP1 cells to IFN-γ irreversibly suspended their growth. The cells stopped dividing and shed off the growth surface (Figure 2D and E).

3.3. Analysis of cell migration :

The monitoring of cell migration revelead differences in the migration rates of HaCaT-MMP1 and HaCaT-C cells. Particularly, HaCaT-KTR cells gradually covered the scratch (Figure 3A). Moreover, either tested Th1 cytokine accelerated their migration compared to untreated HaCaT-KTR cells. In the same time, the migration rates of HaCaT-MMP1 cells treated with either IL17 or TNF, only slightly exceeded the migration rate of untreated HaCaT-KTR cells (Figure 3B). Moreover, either untreated HaCaT-MMP1 or HaCaT-MMP1 treated with IFN-γ mostly remained at the scratch edge for the time of experiment.

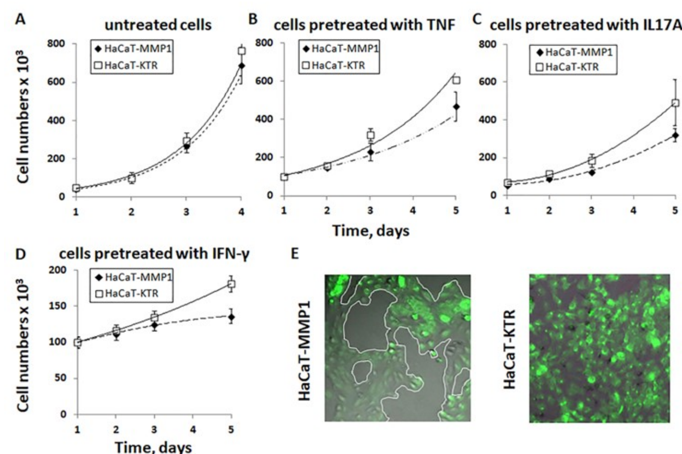

Figure 2 | Proliferation of MMP1-deficient and control HaCaT cells. A – cells not exposed to the cytokines; cells exposed to TNF (B), IL17 (C) and IFN-γ (D); E – Cell survival in the presence of IFN-γ. The cytokines were used at the following concentrations: TNF – 20 ng/mL, IFN-γ – 50 ng/mL; and IL17 – 50 ng/mL. Изменения скорости пролиферации в трансдуцированных эпидермальных кератиноцитах HaCaT. A – клетки, необработанные цитокинами; Клетки, обработанные TNF (B), IL17 (C) и IFN-γ (D); E – гибель клеток при культивировании с IFN-γ. Для проведения экспериментов использовали следующие концентрации цитокинов: TNF – 20 нг/мл, IFN-γ – 50 нг/мл и IL17 – 50 нг/мл

3.4 Analysis of gene expression:

To assess changes in gene expression caused by MMP1 silencing, we selected a pannel of ten genes. Six of these genes were involved into regulation of proliferation (CCNA2, CCND1 and MKI67) and differentiation (IVL, LOR and FLG) of epidermal keratinocytes. The others (KRT17, MMP1, MMP9 and MMP12) were associated with the pathogenesis of disease [12]. Then, we treated HaCaT-KTR and HaCaT-MMP1 cells with one of Th1 cytokines (TNF, IL17 or IFN-γ), obtained cDNA and performed qPCR.

The results of qPCR experiments demonstrated that each cytokine produced a distinct gene expression profile. In HaCaT-MMP1 cells, IFN-γ caused significant changes in expression of all ten genes (Table 1). In the same time, an exposure of these cells to IL17 did not change the expression of IVL and FLG, whereas their treatment with TNF (Table 1) did not cause significant changes in expression of MKI67 and LOR ($p > 0.05$). In addition, the expression of the terminal differentiation markers IVL, LOR and FLG in HaCaT-KTR cells treated with IL17 as well as the expression of CCNA2, MKI67 and KRT17 in HaCaT-KTR cells treated with TNF did not exceed 1.5 times their expression levels in non-stimulated HaCaT cells.

Importantly, some changes in gene expression that we observed were specifically linked to MMP1 silencing. The target gene MMP1 was downregulated in HaCaT-MMP1 cells even when the cells were exposed to high concentrations of proinflammatory cytokines. The expression of the cyclins CCNA2 and CCND1 changed in the opposite direction. Particularly, CCNA2 expression decreased, whereas CCND1 expression increased in HaCaT-MMP1 cells compared to HaCaT-KTR. In turn, the expression of the proliferation marker MKI67 was higher in HaCaT-MMP1 treated with either IFN-γ or IL17. In the same time, treatment of the named cells with TNF did not change MKI67 expression significantly. In addition, IL17 and TNF downregulated KRT17 in HaCaT-MMP1 cells, whereas the differences in KRT17 expression levels between HaCaT-KTR and HaCaT-MMP1 cells treated with IFN-γ were insignificant.

To explain the altered migration and proliferation of HaCaT-MMP1 cells treated with IFN-γ, we also compared the expression of IFN-γ–dependent proapoptotic markers – DAPK1 and the transcription factor IRF8 in both cell lines after their 24h exposure to the named cytokine. We found that both mentioned genes were upregulated in MMP1-deficient cells (Figure 4A). Moreover, HaCaT-MMP1 exhibited higher expression levels of the genes encoding cytokeratins -1, -10 and -14 (Figure 4B), compared to HaCaT-KTR cells.

4. Discussion

In this paper, we explored how MMP1 silencing in epidermal keratinocytes influenced the biological effects of proinflammatory cytokines TNF, IL17 and IFN-γ that play a crucial role in the pathogeneesis of psoriasis. After evaluation of MMP1 expression in HaCaT-MMP1 and HaCaT-KTR cells (Figure 1), we assessed changes in their migration and proliferation rates (Figure 2 and 3,

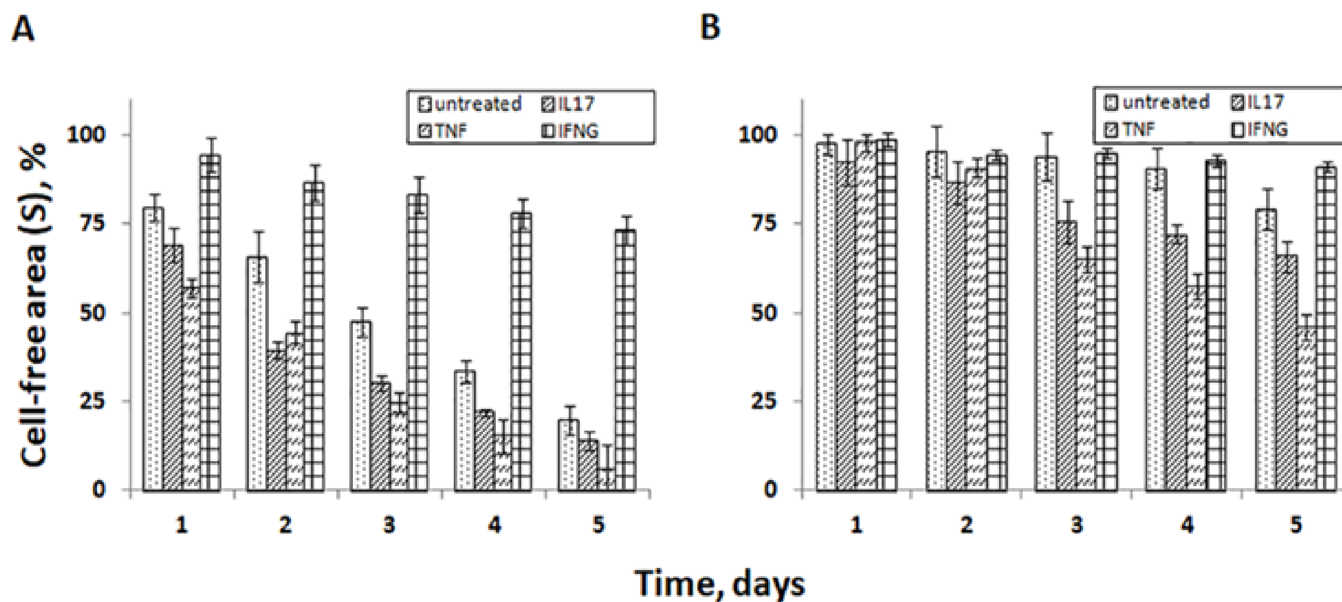

Figure 3 | Migration of MMP1-deficient and control HaCaT cells. Experimental data that reflect the cell migration in real time are represented in linear coordinates: A – HaCaT-KTR; B-HaCaT-MMP1. The cytokines were used at the following concentrations: TNF – 20 ng/mL, IFN-γ – 50 ng/mL; and IL17 – 50 ng/mL. Оценка скоростей миграции в трансдуцированных эпидермальных кератиноцитах HaCaT. Изменения в миграции клеток HaCaT-KTR (A) и HaCaT-MMP1 (B) представлены в линейных координатах. Для проведения экспериментов использовали следующие концентрации цитокинов: TNF – 20 нг/мл, IFN-γ – 50 нг/мл и IL17 – 50 нг/мл.

Table 1 | Gene expression in MMP1-deficient and control cells cultured in the presence of proinflammatory cytokines IFN-γ, TNF and IL17.The cells were treated with one of the mentioned cytokines – IFN-γ (A), IL17 (B) or TNF (C) for 24h. The cytokines were used at the following concentrations: IFN-γ – 50 ng/mL, IL17 – 50 ng/mL or TNF – 20 ng/mL. Gene expression in the parental cell line, i.e. HaCaT cells treated with vehicle control, was referred to as 1-fold relative expression. Изменения экспрессии генов в эпидермальных кератиноцитах HaCaT-MMP1 и HaCaT-MMP1 после их обработки провоспалительными цитокинами IFN-γ, TNF и IL17. Клетки культивировали в присутствии цитокинов IFN-γ (A), IL17 (B) или TNF (C) в течение 24ч. Для проведения экспериментов использовали следующие концентрации цитокинов: TNF – 20 нг/мл, IFN-γ – 50 нг/мл и IL17 – 50 нг/мл.. Уровень экспрессии указанных генов в клетках HaCaT-KTR, обработанных буферным раствором, который не содержал цитокинов, принимали равным единице.

Genes	TNF			IL17			IFN-γ		
	HaCaT-KTR	HaCaT-MMP1	*p*-value	HaCaT-KTR	HaCaT-MMP1	*p*-value	HaCaT-KTR	HaCaT-MMP1	*p*-value
CCNA2	1.11 ± 0.17	0.40 ± 0.06	0.002	0.32 ± 0.05	0.13 ± 0.20	0.020	0.31 ± 0.05	0.24 ± 0.04	0.080
CCND1	0.32 ± 0.05	1.43 ± 0.21	< 0.001	0.13 ±0.02	0.71 ± 0.11	0.107	0.007 ± 0.001	0.02 ± 0.003	< 0.001
MKI67	1.40 ± 0.21	1.07 ± 0.16	0.096	3.80 ± 0.57	5.66 ± 0.89	0.849	1.06 ± 0.16	7.01 ± 1.05	< 0.001
MMP1	1.44 ± 0.01	0.41 ± 0.003	< 0.001	1.02 ± 0.15	0.28 ± 0.04	0.041	3.22 ± 0.48	0.02 ± 0.003	0.003
MMP9	32.15 ± 0.30	25.35 ± 0.24	< 0.001	11.14 ± 1.14	2.29 ± 0.37	0.366	0.57 ± 0.09	1.60 ± 0.24	0.015
MMP12	0.02 ±0.003	0.34 ± 0.05	0.004	7.58 ± 0.65	2.44 ± 0.16	0.161	8.43 ± 1.27	11.10 ±1.67	0.272
KRT17	0.97±0.15	0.35 ± 0.06	0.002	1.64 ± 0.15	0.19 ± 0.02	0.020	2.00 ± .30	2.18 ± 0.33	0.516
IVL	1.83±0.27	1.80 ± 0.27	0.955	1.44 ± 0.24	1.01 ± 0.12	0.115	2.00 ± 0.30	3.98 ± 0.60	0.041
LOR	0.58±0.09	1.35 ± 0.20	0.083	1.15 ± 0.11	1.79 ± 0.17	0.168	0.56 ± 0.08	0.54 ± 0.08	0.898
FLG	0.32±0.02	0.28 ± 0.02	0.372	1.26 ± 0.27	0.89 ± 0.15	0.145	5.77 ± 0.87	13.19 ± 1.98	0.026

respectively). We also compared the expression of genes that are associated with the disease, regulate cell proliferation and the terminal differentiation (Table 1). In addition, we verified the expression of proapoptotic factors DAPK1 and IRF8 and cytokeratins in the cells treated to IFN-γ (Figure 4).

Previously, it was shown that MMP1 silencing changes the morphological characteristics of epidermal keratinocytes [18]. Unlike the cells that expressed scrambled shRNA and preserved a familiar cobblestone-like appearance, MMP1-deficient cells grew in spots, climbed each other and formed layers before they could cover the growth surface. Moreover, the collonies of HaCaT-MMP1 exhibited sharp boundaries, whereas the boundaries of the colonies formed by HaCaT-KTR cells were blurred.

Assuming that MMP1-deficiency influenced the strength of intercellular contacts, we verified how it could affect the cell migration in the presence of proinflammatory cytokines – IL17, TNF and IFN-γ. We found that the ability of MMP1-deficient cells to migrate was impaired and their migration rates did not exceed the migration rate of untreated control cells. For this reason, we assume that MMP1 silencing could be used to suppress structural rearrangements in lesional skin.

Then, we verified how MMP1 silencing could influence the cell proliferation. Surprisingly, when we compared untreated cells we did not see any significant difference between MMP1-deficient and control cells. In contrast, the proliferation rate of MMP1-deficient cells significantly decreased after we treated the cells with either IL-17 or TNF.

Analyzing qPCR data, we found significant changes in MKI67 expression in HaCaT-MMP1 cells treated with IFN-γ. Previously, it was shown that MKI67 is induced in all phases of the cell cycle, except the interphase [20]. In this respect, MKI67 expression level in cultured cells reflects the total number of cells entered the cycle rather than their proliferation rate. For this reason, we also verified whether MMP1-silencing affects the cytokine balance in HaCaT-KTR and HaCaT-MMP1 cells and found that treatment of HaCaT -MMP1 with either TNF or IL17 shifts the balance between CCNA2 and CCND1 in favor of CCND1 (Table 1). To the references, the named cyclins are key regulators of cell cycle. Particularly, CCNA2 controls the transition from G2 to M-phase of cell cycle. In turn, CCND1 is needed for the G1/S transition [21,22]. In this context, we would like to acknowledge that changes in the expression CCNA2 and CCND1 in lesional psoriatic skin are quite opposite to ones that we see in HaCaT-MMP1 cells. Compared to uninvolved skin, the skin samples obtained from lesional skin exhibit a 2 -fold decrease in CCND1 [23] and an 8.7-fold increase in CCNA2 [24] expression. Respectively, a shift in the expression of cyclins that we observe in real-time PCR experiments explains why MMP1-deficienct cells exhibit lower proliferation rate.

However, the most dramatic changes we observe in the presence of IFN-γ. Particularly, we see that MMP1-deficient cells stopped proliferating and detached from the growth surface. To explain this phenomenon, we compared the DAPK1 and IRF8 in the cells treated with IFN-γ.

DAPK1 and IRF8 in the cells treated with IFN-γ. Respectively, we found that their expression levels were higher in HaCaT-MMP1 cells. We also discovered a higher expression of the late differentiation markers loricrin and filaggrin (Table 1) as well as the genes encoding KRT1, -5, -10 and -14. The observed changes in gene expression suggested us that MMP1-silencing could potentially trigger apoptosis and shift the balance between differentiation and proliferation in epidermal keratinocytes toward differentiation.

Notably, the changes in the expression of KRT17 that we observe in response to either IL17 or TNF are opposite to ones that occur in lesional psoriatic skin [25]. In the skin of lab animals, suppression of KRT17 that is often referred to as a "key gene" of psoriasis [26,27] prevents hyperplasia, i.e. thickening of the epidermis due to more intensive cell division and lowering the intensity of inflammatory process. In contrast, an induction of KRT17 stimulates the secretion of Th1 chemokines, such as CXCL5, -9, -10, -11 [26]. For this reason, downregulation of KRT17 caused by MMP1 silencing could be beneficial for the psoriasis patients. Particularly, MMP1 silencing in epidermal keratinocytes could attenuate the inflammatory response and suppress hyperplasia in lesional psoriatic skin.

5. Concluding Remarks

In conclusion, we would like to summarize our key findings:

1. In the cultured epidermal keratinocytes, MMP1-deficiency shifted the balance between differentiation and proliferation toward differentiation. Respectively, MMP1-deficiency can be used to stimulate the terminal differentiation of epidermal keratinocytes in lesional skin.

2. Unlike the cells where MMP1 expression was not affected by shRNA, MMP1-deficient cells were susceptible to apoptosis in the presence of IFN-γ. Respectively, MMP1-deficiency can be used to promote apoptosis in lesional epidermis where IFN-γ is elevated.

3. In addition, MMP1 targeting could be beneficial for psoriasis due to its antiproliferative effect in the presence of proinflammatory cytokines. Respectively, MMP1-deficiency can be used to normalize the proliferation rate of epidermal keratinocytes in diseased skin.

We would also acknowledge that delivery of MMP1 shRNA to diseased skin is a challenging task that requires an optimization of existing delivery systems to make the genetic material of interest (shRNA, genetically modified virions etc.) capable of penetrating the skin barrier.

Заключение

В заключение, мы хотели бы обобщить основные результаты нашей работы.

1. «Нокдаун» MMP1 в культивируемых эпидермальных кератиноцитах приводит к смещению баланса между их дифференцировкой и пролиферацией в сторону дифференцировки. По этой причине, РНК-интерференцию MMP1 можно использовать для того, чтобы стимулировать терминальную дифференцировку эпидермальных кератиноцитов в пораженной болезнью коже.

2. В отличие от клеток с нормальным уровнем экспрессии MMP1 эпидермальные кератиноциты с пониженным уровнем экспрессии этого гена подвержены апоптозу в присутствии IFN-γ. В силу

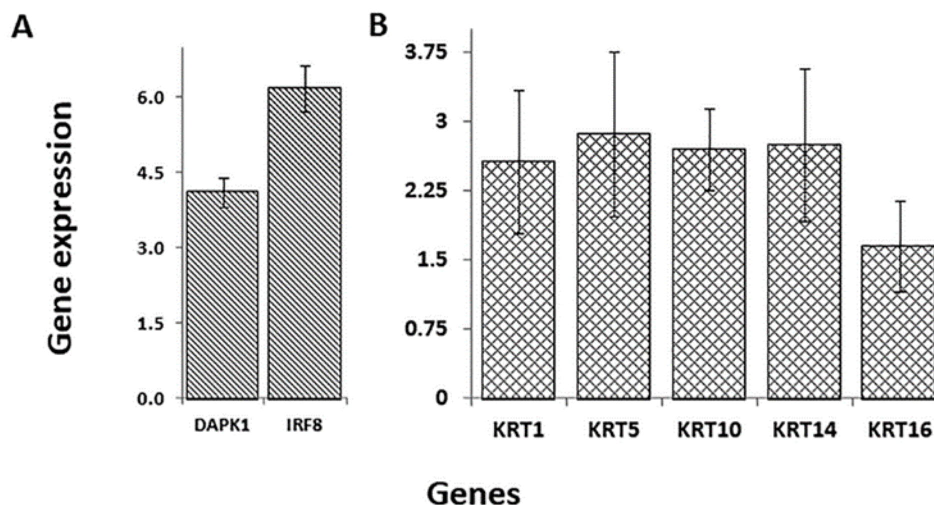

Figure 4 | Migration Expression of proapoptotic factors and cytokeratines in MMP1-deficient cells exposed to IFN-γ. The cells were treated with IFN-γ (50 ng/mL) for 24h. A – expression of proapoptotic factors and B – expression of cytokeratins. Gene expression in HaCaT-KTR cells treated with IFN-γ, was referred to as 1-fold relative expression. Уровень экспрессии маркеров апоптоза и цитокератинов в эпидермальных кератиноцитах HaCaT-MMP1, обработных IFN-γ. Клетки культивировали в присутствии IFN-γ (50 нг/мл) в течение 24ч. Экспрессия маркеров апоптоза (A) и генов, кодирующих цитокератины (B). Уровень экспрессии указанных генов в клетках HaCaT-KTR, обработанных IFN-γ, принимали равным единице.

этого, снижение уровня экспрессии MMP1 можно использовать для того, чтобы инициировать апоптоз в псориатическом эпидермисе, где концентрация IFN-γ превышает нормальные физиологические значения.

3. Наконец, РНК-интерференция MMP1 может иметь клиническое значение для псориаза, поскольку присутствие провоспалительных цитокинов в культуральной среде приводит к снижению скорости пролиферации клеток с дефицитом MMP1. Соответственно, «нокдаун» MMP1 можно использовать для того, чтобы снизить скорость пролиферации эпидермальных кератиноцитов in vivo.

Мы также хотели бы отметить, что в настоящее время проведение РНК-интерференции в терапевтических целях все еще является трудновыполнимой задачей, поскольку эпидермис, являясь естественным барьером на пути чужеродных биомолекул, эффективно мешает их проникновению в кожу. Решение этой задачи потребует разработки более эффективных способов трансфекции клеток, находящихся в коже больного

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