Вавиловский журнал генетики и селекции. 2018;22(4):425-432
DOI 10.18699/VJ18.378

RNAi-mediated silencing of matrix metalloproteinase 1 in epidermal keratinocytes influences the biological effects of interleukin 17A

J.A. Mogulevtseva[1], A.V. Mezentsev[2] ✉, S.A. Bruskin[2]

[1] Russian State Agrarian University – Moscow Timiryazev Agricultural Academy, Moscow, Russia
[2] N.I. Vavilov Institute of General Genetics RAS, Moscow, Russia

Matrix metalloproteinases (MMPs) are important for the pathogenesis of psoriasis and other autoimmune disorders. In the extracellular matrix, accumulation of proinflammatory cytokines, such as interleukin 17A (IL-17A), leads to induction of several *MMPs*, including *MMP1*. MMPs change the composition and other properties of the extracellular matrix. These changes facilitate tissue remodeling and promote the development of psoriatic plaques. The aim of this study was to explore how *MMP1* silencing might influence the biological effects of IL-17A on migration and proliferation of human epidermal keratinocytes and the expression of genes involved in their division and differentiation. The experiments were performed with MMP1-deficient and control epidermal keratinocytes, HaCaT-MMP1 and HaCaT-KTR, respectively. Cell proliferation and migration were assessed by comparative analysis of the growth curves and scratch assay, respectively. To quantify cell migration, representative areas of cell cultures were photographed at the indicated time points and compared to each other. Changes in gene expression were analyzed by real-time PCR. The obtained results demonstrated that *MMP1* silencing in the cells treated with IL-17A resulted in downregulation of *MMP9* and *-12*, *FOSL1*, *CCNA2*, *IVL*, *KRT14* and *-17* as well as upregulation of *MMP2*, *CCND1* and *LOR*. Moreover, *MMP1* silencing led to a decrease in cell proliferation and an impairment of cell migration. Thus, MMP1-deficiency in epidermal keratinocytes can be beneficial for psoriasis patients that experience an accumulation of IL-17 in lesional skin. Knocking *MMP1* down could influence migration and proliferation of epidermal keratinocytes *in vivo*, as well as help to control the expression of *MMP1*, *-2*, *-9* и *-12*, *CCNA2*, *CCND1*, *KRT14* and *-17* that are crucial for the pathogenesis of psoriasis.

Key words: matrix metalloproteinase 1; psoriasis; interleukin 17; small hairpin RNA; gene silencing.

HOW TO CITE THIS ARTICLE:
Mogulevtseva J.A., Mezentsev A.V., Bruskin S.A. RNAi-mediated silencing of matrix metalloproteinase 1 in epidermal keratinocytes influences the biological effects of interleukin 17A. Vavilovskii Zhurnal Genetiki i Selektsii = Vavilov Journal of Genetics and Breeding. 2018;22(4):425-432. DOI 10.18699/VJ18.378

Received 22.11.2017
Accepted for publication 05.02.2018
© AUTHORS, 2018

e-mail: mesentsev@vigg.ru

Особенности протекания РНК-интерференции матриксной металлопротеиназы 1 в эпидермальных кератиноцитах, обработанных интерлейкином 17А

Ю.А. Могулевцева[1], А.В. Мезенцев[2] ✉, С.А. Брускин[2]

[1] Российский государственный аграрный университет – МСХА им. К.А. Тимирязева, Москва, Россия
[2] Институт общей генетики им. Н.И. Вавилова Российской академии наук, Москва, Россия

Матриксные металлопротеиназы (ММП) играют важную роль в патогенезе псориаза, а также ряда других аутоиммунных заболеваний. Накопление интерлейкина 17А (ИЛ-17А) и других провоспалительных цитокинов в межклеточном матриксе приводит к индукции генов некоторых матриксных металлопротеиназ, в частности *MMP1*. Рост протеолитической активности в межклеточном матриксе меняет его состав и свойства, а также способствует структурной реорганизации пораженного болезнью участка кожи. Структурная реорганизация, в свою очередь, приводит к изменению внешнего облика кожных покровов и образованию псориатических бляшек. Целью данной работы было исследовать влияние РНК-интерференции *MMP1* на биологические эффекты ИЛ-17А, такие как способность данного цитокина стимулировать миграцию и пролиферацию эпидермальных кератиноцитов человека, а также регулировать экспрессию генов, которые играют важную роль в процессе дифференцировки данного типа клеток. В работе использовали иммортализованные эпидермальные кератиноциты с «нокдауном» *MMP1* и без него – HaCaT-ММП1 и HaCaT-КТР соответственно. Для оценки пролиферации клеток сопоставляли кривые их роста. Миграцию клеток оценивали путем сравнения репрезентативных фотографических изображений, которые были получены через равные промежутки времени. Изменения в экспрессии генов анализировали методом ПЦР в режиме реального времени. Согласно полученным результатам, в клетках, обработанных ИЛ-17А, РНК-интерференция *MMP1* приводит к уменьшению экспрессии *MMP9* и *MMP12*, *FOSL1*, *CCNA2*, *IVL*, *KRT14* и *KRT17*, а также к увеличению экспрессии *MMP2*, *CCND1* и *LOR*. «Нокдаун» *MMP1* замедляет процесс миграции клеток и приводит к снижению скорости их пролиферации. Таким образом, проведенное нами

исследование показало, что в присутствии ИЛ-17А РНК-интерференция *MMP1* обладает потенциальным терапевтическим эффектом, который может быть использован при лечении псориаза. «Нокдаун» ММП1 позволяет воздействовать на пролиферацию и миграцию клеток, а также контролировать экспрессию важных для патогенеза болезни генов (*MMP1*, *MMP2*, *MMP9* и *MMP12*, *CCNA2*, *CCND1*, *KRT14* и *KRT17*).

Ключевые слова: матриксная металлопротеиназа 1; псориаз; интерлейкин 17; малая ингибирующая РНК; РНК-интерференция.

Plaque psoriasis (psoriasis vulgaris) is one of the most abundant chronic non-infectious skin conditions (Greb et al., 2016). According to the World Health Organization, the prevalence of psoriasis worldwide is ~3% (Michalek et al., 2017). In mainland Russia, the prevalence of psoriasis is ~1.9 % (Khamaganova et al., 2015). The most distinctive hallmark of psoriasis is the appearance of thick scaly plaques on the patient's skin. In 21.1 % and 5.7 % of cases, respectively, the disease targets nails and joints (Rukavishnikova, 2009; Mishina et al., 2013). Moreover, psoriasis is often accompanied by comorbidities, such as cardiovascular disease, type II diabetes and atherosclerosis (Batyrshina, Sadykova, 2014).

At the molecular level, the development of psoriatic plaques leads to differential expression of thousands of genes (Zolotarenko et al., 2016), including the genes involved in proliferation and the terminal differentiation of epidermal keratinocytes. In lesional skin, *KRT1*, *KRT5* and *KRT10* are downregulated (Rao et al., 1996; Jin, Wang, 2014), whereas *KRT14, KRT16,* and *KRT17* are upregulated (Al Robaee, 2010; Jin, Wang, 2014). The late differentiation markers loricrin (*LOR*) and filaggrin (*FLG*) are expressed there at a lower level. In contrast, the expression level of the early differentiation marker involucrin (*IVL*) is higher (Soboleva et al., 2014a). The proliferation marker *MKI67* (Yazici et al., 2005) and cyclin A2 (Manczinger, Kemény, 2013) are upregulated, while cyclin D1 is downregulated (Reischl et al., 2007).

The proinflammatory cytokine interleukin 17A (IL-17A) plays a key role in the pathogenesis of psoriasis. Specifically, IL-17A contributes to the inflammatory response by inducing the chemokines *CCL20*, *CXCL1*, *CXCL2*, and *CXCL8*. Their secretion to the extracellular matrix promotes the migration of activated immune cells to lesional epidermis. In turn, the accumulation of immune cells in lesional skin stabilizes there the inflammatory process (Seo et al., 2017).

This concept fits well with emerging results from clinical trials with IL-17A-specific antibodies (Canavan et al., 2016). According to the clinical data, ~85–90 % of patients with moderate and severe psoriasis achieve $PASI_{75}$ after completion of the therapy. Moreover, the tested IL-17A-specific antibodies exhibit low immunogenicity. On the other hand, these medicines affect the patients' ability to respond to pathogens. In addition, people who experience allergic reactions or were diagnosed with Chron's disease and ulcerative colitis are advised from taking injections of IL-17A-specific antibodies. In this respect, an approval of IL-17A-specific antibodies for use in clinical practice does not diminish further efforts to find safer and even more efficient treatment options for psoriasis, such as a modulation of gene expression with specific shRNA.

In the lab, our research is focused on matrix metalloproteinases, such as matrix metalloproteinase 1 (MMP1), and their role in psoriasis. Previously, we demonstrated that changes of *MMP1*, *MMP9* and *MMP12* expression levels coincide with flaring of the disease and correlate with the disease severity (Starodubtseva et al., 2011). In this paper, we are exploring how *MMP1* silencing with specific shRNA might influence migration and proliferation of epidermal keratinocytes treated with IL-17A. We also wanted to reveal whether knocking *MMP1* down affected the expression of genes involved in the pathogenesis of psoriasis.

Materials and methods

Cell culturing. The experiments were performed with MMP1-deficient and control immortalized epidermal keratinocytes, HaCaT-MMP1 and HaCaT-KTR, respectively. The mentioned cell lines were obtained as described previously (Mogulevtseva, Mezentsev, 2017). The cells were cultured in DMEM medium, supplemented with 5 % embryonic calf serum, L-glutamine (PanEco, Russia) and antibiotic-antimycotic (ThermoFisher Scientific, USA). The medium was replaced every other day. Once the cells reached 70–75 % confluence, they were seeded into new dishes at a ratio of 1:5. The cells were counted with a hemocytometer.

Preparation of total RNA. The total RNA was purified with TRIZOL (ThermoFisher Scientific) as described previously (Chomczynski, Mackey, 1995). Purity and integrity of the obtained RNA were verified using non-denaturing 1.5 % agarose gel electrophoresis. A Qubit RNA BR Assay Kit (ThermoFisher Scientific) was used to quantify RNA according to the manufacturer's protocol.

Real-time PCR. Before the experiment, RNA was converted to cDNA using an MMLV RT kit (Evrogen, Russia). The experiments were carried out in the Eco real-time PCR system (Illumina, USA) according to the instructions supplied by the manufacturer. The primers used in this study were taken from the NCBI Probe database (NCBI Probe, 2015). The *ACTB* assay was used as an endogenous control. The results were analyzed using preinstalled software supplied by the manufacturer. Each probe was run in triplicates. Overall, three independent experiments were performed.

Proliferation assay. To assess the cell proliferation rate, cells were plated at 40,000 cells/well in 6-well plates. To obtain cell suspensions, randomly chosen samples were treated with 0.25 % trypsin-EDTA (PanEco) on a daily basis. The cell

suspensions were stained with 0.2 % trypan blue and counted with a hemocytometer. Then, the cell counts were plotted against the incubation time to generate the growth curves in linear coordinates. To assess changes in cell proliferation rates, the data were represented in semilogarithmic coordinates and subjected to linear regression analysis. Slops of these lines served us as the estimates of the cell proliferation rates. Overall, three independent experiments were performed.

Scratch assay. To assess cell migration, the designated cell lines were cultured until confluence. The cell monolayer was scratched with a pipette tip to form a 1.25 mm-wide cell-free area across the center of the well. Then, the remaining cells were cultured for 5–6 days and the most representative cell-free areas were photographed on a daily basis. These areas were quantified using the "Freehand selection" tool of "ImageJ" freeware (Schindelin et al., 2015).

Statistical analysis. The data obtained were represented as the mean ± standard error (m ± SE). The statistical differences between the means were assessed by a one-way analysis of variances. If p values were less than 0.05, the means were considered to be significantly different.

Results

Influence of *MMP1* silencing on gene expression

Culturing HaCaT-KTR and HaCaT-MMP1 cells in the presence of IL-17A resulted in a differential expression of matrix metalloproteinases, cytokeratins, proliferation markers and terminal differentiation markers of epidermal keratinocytes (Fig. 1).

Particularly, the exposure of HaCaT-KTR cells to IL-17A led to upregulation of *MMP9* and *MMP12* (11.15 ± 1.67 and 7.58 ± 1.14, respectively) and downregulation of *MMP2* (0.37 ± 0.05). In the same time, their expression levels in HaCaT-MMP1 cells were 2.29 ± 0.34, 2.44 ± 0.37 and 0.98 ± 0.15, respectively (Fig. 1, *a*). Moreover, *MMP1* expression in MMP1-deficient cells remained relatively low (0.28 ± 0.04), despite the cells were exposed to a high concentration of IL-17A.

The expression of the cell proliferation marker *MKI67* was increased in both cell lines (3.80 ± 0.57 in HaCaT-KTR cells and 5.66 ± 0.85 in HaCaT-MMP1 cells, $p = 0.36$). In contrast, cyclins *CCNA2* and *CCND1* were downregulated (Fig. 1, *b*). Particularly, *CCNA2* expression levels were 0.32 ± 0.05 in HaCaT-KTR cells and 0.13 ± 0.02 in HaCaT-MMP1 cells, while *CCND1* expression levels were 0.13 ± 0.02 and 0.71 ± 0.11, respectively.

Moreover, we observed slight changes in the expression levels of the terminal differentiation markers in HaCaT-KTR cells. Particularly, the expression levels of *IVL*, *LOR* and *FLG* were 1.44 ± 0.13, 1.15 ± 0.11 and 1.26 ± 0.27, respectively (Fig. 1, *c*), whereas the expression levels of the mentioned genes in HaCaT-MMP1 cells were 1.01 ± 0.11 (*IVL*), 1.79 ± 0.17 (*LOR*) and 1.89 ± 0.14 (*FLG*). In HaCaT-KTR cells, the expression levels of cytokeratins did not exceed 1.5 compared to the negative control, except *KRT14* and *KRT17*, which were upregulated (1.86 ± 0.14

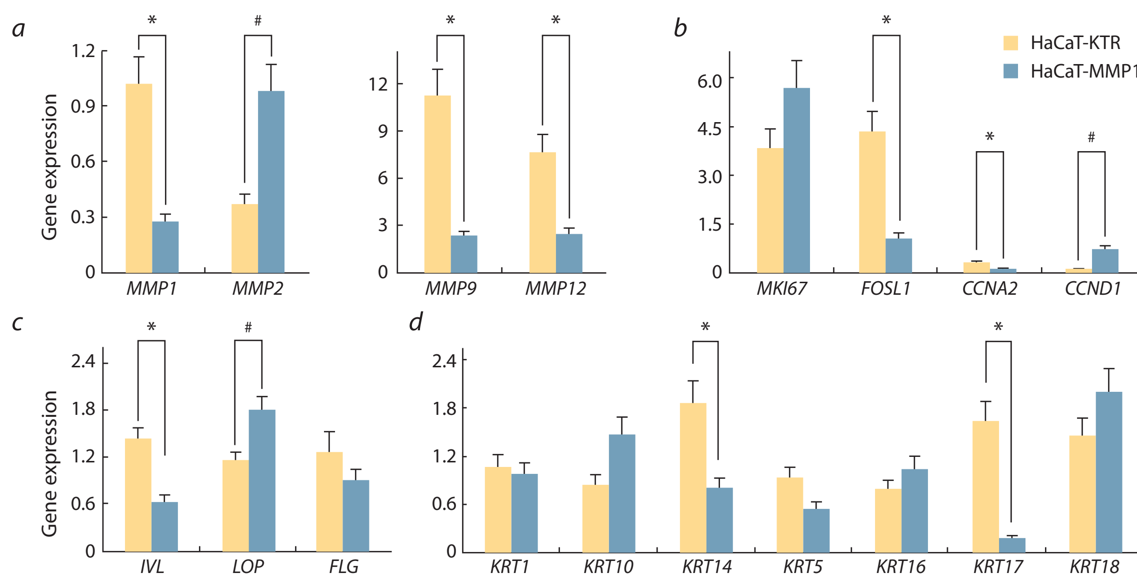

Fig. 1. qPCR analysis of gene expression in human epidermal keratinocytes HaCaT-MMP1 and HaCaT-KTR treated with IL-17A. The data presented in the figure describe changes in the expression of matrix metalloproteinases (*a*), proliferation markers (*b*), terminal differentiation markers of epidermal keratinocytes (*c*) and cytokeratins (*d*).

The cells were treated with 50 ng/mL IL-17A. In the probes, the measurements were normalized to the level of *ACTB*. The gene expression levels in untreated HaCaT-KTR cells were considered equal to 1. The symbols '#' and '*' were used to mark the genes those expression levels were significantly higher and lower, respectively, in HaCaT-MMP1 than in HaCaT-KTR ($p < 0.05$, $n = 3$).

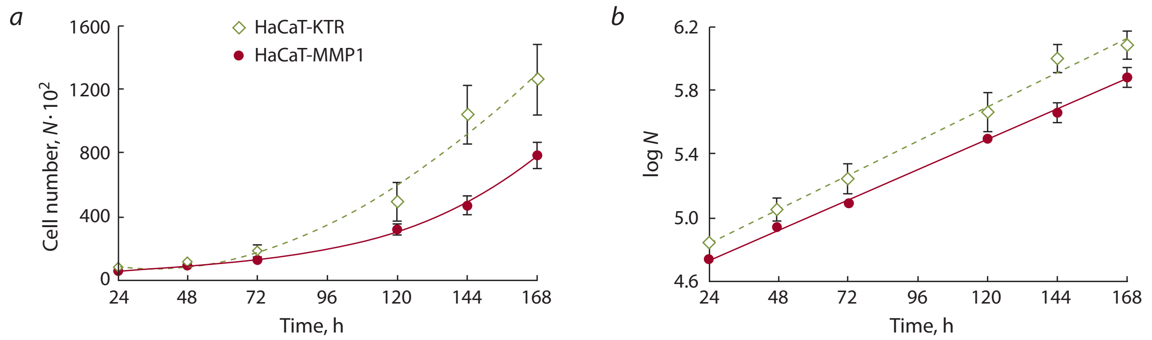

Fig. 2. Influence of MMP1 silencing on the proliferation of HaCaT-KTR and HaCaT-MMP1 cells treated with IL-17A. Experimental curves that reflected the cell growth in real time were plotted in linear (*a*) and semilogarithmic (*b*) coordinates. HaCaT-MMP1 and HaCaT-KTR cells were treated with 50 ng/mL IL-17A for the indicated periods of time.

Details on cell culturing are provided in the section "Materials and methods".

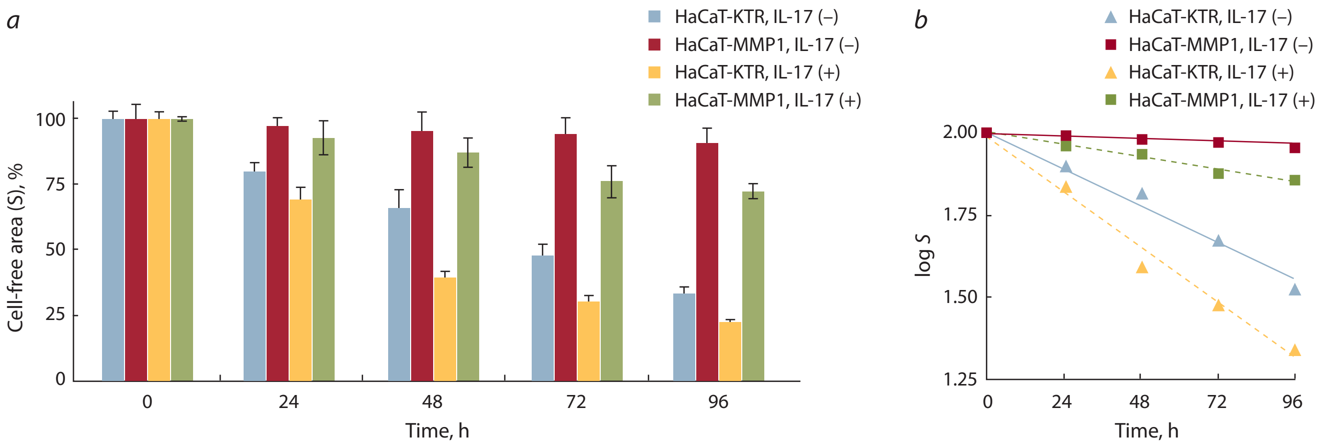

Fig. 3. Influence of *MMP*1 silencing on migration of human epidermal keratinocytes treated with IL-17A. In the figure, the obtained experimental data were represented in linear (*a*) and semilogarithmic (*b*) coordinates.

The cells were exposed to IL-17A (50 ng/mL) for the indicated periods of time, as described in the section "Materials and methods".

and 1.64 ± 1.15, respectively). In contrast, *KRT17* was significantly downregulated in HaCaT-MMP1 (0.19 ± 0.02) compared to HaCaT-KTR cells.

Influence of *MMP1* silencing on cell proliferation

Comparative analysis of the growth curves revealed that HaCaT-MMP1 and HaCaT-KTR cells remained in the active growth phase for the time of the experiment (Fig. 2). The time-dependences of cell growth in linear coordinates (Fig. 2, *a*) suggested that the cells grew monotonously, i.e. for the time of observation, the growth curves did not reach saturation. At the same time, a semilog transformation of the growth curves (Fig. 2, *b*) revealed a high correlation between time and cell numbers ($r > 0.99$; $p < 0.001$). Furthermore, *MMP1* silencing caused a delay in cell growth (Fig. 2, *a*). According to the results of linear regression, the proliferation rates of HaCaT-KTR and HaCaT-MMP1 cells were 0.0090 ± 0.0003 and 0.0078 ± 0.0003, respectively.

Influence of *MMP1* silencing on cell migration

Analysis of cell migration showed that the motility of HaCaT-MMP1 was significantly impaired compared to HaCaT-KTR cells (Fig. 3, *a*). In contrast, exposure of both cell lines to IL-17A stimulated cell migration. In this respect, *MMP1*-deficiency resulted in an 11.48-fold decrease of the migration constant, whereas exposure of HaCaT-KTR and HaCaT-MMP1 cells to IL-17A caused 1.43- and 3.63-fold increases of the corresponding migration constants (Fig. 3, *b*).

Discussion

IL-17A is one of proinflammatory cytokines that are implicated in the pathogenesis of various autoimmune diseases including psoriasis (Korotaeva, 2016). In psoriasis, IL-17A is accumulated in lesional skin. After secretion by T-helper 17 (T_{h17}) immune cells into the extracellular matrix, IL-17A activates the IL-17RA receptor, which is located in the plasma membrane of immunocytes, such as macrophages, and resident skin cells primarily keratinocytes, dendritic cells and fibroblasts. The interaction of IL-17A with IL-17RA results in the induction of various proinflammatory cytokines (TNF, IL-1β, and IL-6, etc.) and chemokines (IL-8, CXCL1, CXCL2 and CCL20) (Mills, 2008; Seo et al., 2017). The induced cytokines influence proliferation and differentiation of epidermal keratinocytes, whereas chemokines promote the

migration of immune cells, such as neutrophils and monocytes to the skin. The former causes the development of psoriatic plaques. The latter stabilizes the inflammatory response in psoriatic plaques.

The concentration of IL-17A in the blood serum of healthy volunteers usually does not exceed 10 pg/mL (Shilova et al., 2015). In contrast, the biological effects of IL-17A in cultured epidermal keratinocytes and fibroblasts, such as activation of protein kinases (Peric et al., 2008, 2009), induction of proinflammatory cytokines (Tohyama et al., 2009; Cho et al., 2012) or influencing cell proliferation (Ma, Jia, 2016) require an incubation of cells in the presence of 10–100 ng/mL IL17-A. Presumably, this difference can be explained by the fact that in psoriasis, T_{h17} cells, which are the main source of IL17-A in the body, are predominantly located in the inflamed tissue where they are needed to stabilize the inflammatory response. For this reason, we treated epidermal keratinocytes with 50 ng/mL IL-17A (Starodubtseva et al., 2011).

Culturing HaCaT-KTR and HaCaT-MMP1 cells in the presence of IL-17A, we anticipated that the genes involved in the terminal differentiation and proliferation of epidermal keratinocytes would be differentially expressed. Primarily, we expected to see changes in gene expression similar to ones that occur during the development of psoriatic plaques. In this respect, changes in the expression of *IVL*, *MKI67, KRT14* and *KRT17* that we observed in HaCaT-KTR cells, i.e. the cell line that expressed scrambled shRNA, did not surprise us (see Fig. 1). We also wanted to explore how *MMP1* silencing could influence the expression of genes that were implicated in the pathogenesis of psoriasis. Notably, upregulation of *MKI67*, *MMP9* and *MMP12* as well as downregulation of *CCNA2* and *CCND1* occur in both cell lines, i.e. it is unlikely that they are caused by *MMP1*-silencing (see Fig. 1, *a* and *b*). In the same time, lower expression levels of *MMP9* and *MMP12* as well as a higher expression level of *MMP2* in HaCaT-MMP1 suggest that the expression of *MMP1*-specific shRNA could help to control MMPs expression in lesional psoriatic skin (Nair et al., 2009). Moreover, changes in the expression of the terminal differentiation markers, such as downregulation of *IVL* (p = 0.05) and upregulation of *LOR* (p = 0.03) in HaCaT-MMP1 cells (see Fig. 1, *c*) are also opposite to ones that occur in lesional psoriatic skin. However, we did not discover significant differences in *FLG* expression between HaCaT-MMP1 and HaCaT-KTR (p = 0.29).

Notably, exposure of both cell lines to IL-17A does not cause significant changes in the expression of many cytokeratins (see Fig. 1, *d*). This can be explained by the fact that HaCaT cells, i.e. the cell line that was used to generate HaCaT-KTR and HaCaT-MMP1 cells, and primary epidermal keratinocytes react differentially to treatment with IL-17A. Particularly, culturing the primary cells in the presence of 50 ng/mL IL-17A results in downregulation of *KRT10*, *FLG*, *LOR* and *IVL* (Noh et al., 2010), whereas treatment of HaCaT with the same concentration of IL-17A does not cause any significant changes in the expression of the named genes (Seo et al., 2012). In contrast, we report here that the incubation of HaCaT-KTR cells with IL-17A results in upregulation of *KRT14* and *KRT17*. These changes in gene expression are similar to ones that occur in lesional psoriatic skin (Nair et al., 2009). However, it is even more important that *MMP1* silencing downregulates both genes. For instance, it results in more than a 10-fold downregulation of *KRT17*. In the published papers, *KRT17* is often referred to as a "key gene" of psoriasis (Al Robaee, 2010; Jin, Wang, 2014). Suppression of *KRT17* in the skin of lab animals prevents hyperplasia, i.e. thickening of the epidermis due to more intensive cell division and lowering the intensity of the inflammatory process. In contrast, induction of *KRT17* stimulates the secretion of T_{h1} chemokines, such as CXCL5, CXCL9, CXCL10 and CXCL11 (Al Robaee, 2010). In this respect, we assume that *MMP1* silencing could be beneficial for psoriasis patients to attenuate the inflammatory response and suppress hyperplasia in lesional psoriatic skin.

Comparative analysis of gene expression in HaCaT-MMP1 and HaCaT-KTR cells treated with IL-17A reveals that HaCaT-MMP1 cells express less *CCNA2* and more *CCND1* (see Fig. 1, *b*). However, differences in the expression of the proliferation marker *MKI67* between these two cell lines are insignificant (p = 0.36). According to the previously published data, *CCND1* is required for the transition from G_1-phase of the cell cycle to S-phase, whereas *CCNA2* is needed for the transition from G_2-phase to M-phase (Matsushime et al., 1992; Pagano et al., 1992). Furthermore, the changes in the expression of *CCND1* and *CCNA2* that the others observe in psoriatic lesional skin are opposite to the ones we see in *MMP1*-deficient cells. Particularly, comparative analysis of skin samples obtained from lesional and uninvolved skin reveals a two-fold decrease for *CCND1* (Reischl et al., 2007) and an 8.7-fold increase for *CCNA2* (Manczinger, Kemény, 2013) in lesional skin. This shift in the cytokine balance is also in line with the results of our cell proliferation assay (see Fig. 2). According to the obtained data, HaCaT-MMP1 cells exhibit a 1.15-fold decrease in the constant of proliferation compared to HaCaT-KTR cells. At the same time, IL-17A does not stimulate proliferation of HaCaT cells *in vitro* (Soboleva et al., 2014b). Hence, we propose that *MMP1* silencing could also exert an antiproliferative effect in lesional psoriatic skin that accumulates IL-17A.

Moreover, culturing HaCaT-KTR cells in the presence of IL-17A results in differential expression of the matrix metalloproteinases (see Fig. 1, *a*) *MMP2* (0.37 ± 0.05), *MMP9* (11.15 ± 1.67) and *MMP12* (7.58 ± 1.14). To the reference, similar changes in the expression of the named metalloproteinases occur in lesional psoriatic skin. For instance, one of the previous studies performed in our lab revealed that in lesional psoriatic skin, *MMP2* was downregulated (0.77 ± 0.23), whereas *MMP9* and *MMP12* were upregulated (4.2±0.65-fold and 17.25±5.80-fold, respectively) compared to uninvolved skin (Starodubtseva et al., 2011). In this study, we show that *MMP2*, *MMP9* and *MMP12* expression levels in MMP1-deficient cells (0.98 ± 0.15, 2.29 ± 0.34 and 2.44 ± 0.37, respectively) are comparable to the negative control, i.e. HaCaT-KTR, untreated with IL-17A. In this respect, the obtained results suggest that *MMP1* silencing could be used *in vivo* to control the expression of the mentioned matrix metalloproteinases, primarily *MMP9* and *MMP12*.

In this paper, we also report that IL-17A stimulates migration of both HaCaT-KTR and HaCaT-MMP1 cells (see Fig. 3). According to the published data, IL-17A promotes the

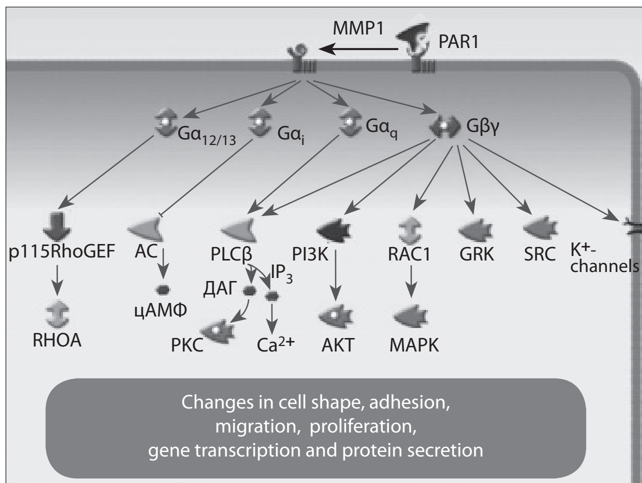

Fig. 4. The proposed intracellular signalling mechanisms underlying MMP1-induced activation of the PAR1 receptor.

The following molecules participating in MMP1-induced activation of PAR1 are located downstream of G-coupled proteins. The proteins: p115RhoGEF, RHO guanine nucleotide exchange factor 1; AC, adenylyl cyclase; PLCβ, β isoform of phospholipase C; PI3K, phosphoinositide 3-kinase; RAC1, ras-related C3 botulinum toxin substrate 1; GRK, G protein-coupled receptor kinase; SRC, proto-oncogene c-SRC; RHOA, RAS homologue gene family, member A; PKC, protein kinase C; AKT, AKT kinase or protein kinase B; and MAPKs, mitogen-activated protein kinases. The selected products of their catalytic activity: DAG, diacylglycerol; IP_3, inositol trisphosphate; and cAMP, cyclic adenosine monophosphate.

migration of fibroblasts (Wu et al., 2014) and blood vessel endothelial cells (Vegfors et al., 2016). However, the influence of IL-17A on migration of epidermal keratinocytes was not previously reported. As it was previously discussed, the mobility of epidermal keratinocytes depends on the expression levels of matrix metalloproteinases, primarily *MMP1*, *MMP3*, *MMP9*, and *MMP13* (Mezentsev et al., 2014). Moreover, it can be increased by treatment of the cells with tumor necrosis factor (TNF) due to the ability of TNF to induce *MMP9* (Scott et al., 2004). The authors of the cited paper report that TNF induces cell migration in a dose-dependent manner. Moreover, treatment of the cells with antibodies specific to either MMP9 or TNF significantly slows the migration down. In turn, we report that culturing the cells in the presence of IL-17A induces *MMP9* and *MMP12*. According to our data, HaCaT-KTR cells where *MMP9* is induced gain a higher motility, compared to HaCaT-MMP1 cells where *MMP9* is downregulated (see Fig. 1, *a*).

In conclusion, we would like to acknowledge that the results of this study suggest reconsidering the traditional role of MMP1 as a proteolytic enzyme. Obviously, the influence of *MMP1* silencing on the expression of cyclins and cytokeratins as well as the negative effect on cells proliferation can be explained by the direct participation of this enzyme in intracellular signalling pathways. First, this is possible due to some products of the MMP1-catalyzed reaction known as matrikins mediate the signalling mechanisms (Wells et al., 2015). Secondly, some matrix metalloproteinases, such as MMP1, directly interact with PAR receptors (Boire et al., 2005). Following the binding to the G-protein coupled PAR1 receptor (Fig. 4), MMP1 cuts N-terminal peptide. Then, this peptide binds to the receptor as a ligand and they form an active ligand-receptor complex. On the cytoplasmic side of the cell membrane, the complex interacts with one of G-proteins ($G\alpha_{12/13}$, $G\alpha_1$, $G\alpha_q$ и $G\beta\gamma$). Each of these proteins activates a unique pattern of signalling pathways that may result in a different outcome for the cell. Although the factors that influence the interaction of PAR receptors with particular G-proteins still need to be studied, activation of the same receptor in different physiological conditions may cause a shape change, changes in cell behavior, induction of certain genes or activation of protein secretion. Importantly, similar changes are also observed in the pathogenesis of psoriasis. As we believe, the key findings of this paper open an opportunity for the creation of a new therapeutic approach that can be used for psoriasis. In this respect, we would like to mention that new recombinant viruses were proposed to deliver the desired genes to the diseased organs and tissues and several therapeutic approaches were proposed to treat human genetic disorders (Hacein-Bey-Abina et al., 2002; Bainbridge et al., 2008). Moreover, viral transfection was successfully used to treat psoriasis in humanized animals (Jakobsen et al., 2009).

Acknowledgments

The authors are grateful to Prof. E.S. Piruzian for critical analysis of the manuscript and fruitful discussion of the obtained results.

Conflict of interest

The authors declare no conflict of interest.

References

Al Robaee A.A. Molecular genetics of Psoriasis (Principles, technology, gene location, genetic polymorphism and gene expression). Int. J. Health Sci. (Qassim). 2010;4(2):103-127.

Bainbridge J.W., Smith A.J., Barker S.S., Robbie S., Henderson R., Balaggan K., Viswanathan A., Holder G.E., Stockman A., Tyler N., Petersen-Jones S., Bhattacharya S.S., Thrasher A.J., Fitzke F.W., Carter B.J., Rubin G.S., Moore A.T., Ali R.R. Effect of gene therapy on visual function in Leber's congenital amaurosis. N. Engl. J. Med. 2008;358(21):2231-2239. DOI 10.1056/NEJMoa0802268.

Batyrshina S.V., Sadykova F.G. Comorbid conditions in patients with psoriasis. Prakticheskaya Meditsina = Practical Medicine. 2014;8:32-35. (in Russian)

Boire A., Covic L., Agarwal A., Jacques S., Sherifi S., Kuliopulos A. PAR1 is a matrix metalloprotease-1 receptor that promotes invasion and tumorigenesis of breast cancer cells. Cell. 2005;120(3):303-313. DOI 10.1016/j.cell.2004.12.018.

Canavan T.N., Elmets C.A., Cantrell W.L., Evans J.M., Elewski B.E. Anti-IL-17 medications used in the treatment of plaque psoriasis and psoriatic arthritis: a comprehensive review. Am. J. Clin. Dermatol. 2016;17(1):33-47. DOI 10.1007/s40257-015-0162-4.

Cho K.A., Suh J.W., Lee K.H., Kang J.L., Woo S.Y. IL-17 and IL-22 enhance skin inflammation by stimulating the secretion of IL-1β by keratinocytes via the ROS-NLRP3-caspase-1 pathway. Int. Immunol. 2012;24(3):147-158. DOI 10.1093/intimm/dxr110.

Chomczynski P., Mackey K. Short technical reports. Modification of the TRI reagent procedure for isolation of RNA from polysaccharide and proteoglycan-rich sources. BioTechniques. 1995;19(6):942-945.

Goldminz A.M., Elder J.T., Lebwohl M.G., Gladman D.D., Wu J.J., Mehta N.N., Finlay A.Y., Gottlieb A.B. Psoriasis. Nat. Rev. Dis.

Primers. 2016;2:16082. DOI 10.1038/nrdp.2016.82.

Hacein-Bey-Abina S., Le Deist F., Carlier F., Bouneaud C., Hue C., De Villartay J.P., Thrasher A.J., Wulffraat N., Sorensen R., Dupuis-Girod S., Fischer A., Davies E.G., Kuis W., Leiva L., Cavazzana-Calvo M. Sustained correction of X-linked severe combined immunodeficiency by ex vivo gene therapy. N. Engl. J. Med. 2002;346(16): 1185-1193. DOI 10.1056/NEJMoa012616.

Jakobsen M., Stenderup K., Rosada C., Moldt B., Kamp S., Dam T.N., Jensen T.G., Mikkelsen J.G. Amelioration of psoriasis by anti-TNF-α RNAi in the xenograft transplantation model. Mol. Ther. 2009;17(10):1743-1753. DOI 10.1038/mt.2009.141.

Jin L., Wang G. Keratin 17: a critical player in the pathogenesis of psoriasis. Med. Res. Rev. 2014;34(2):438-454. DOI 10.1002/med.21291.

Khamaganova I.V., Almazova A.A., Lebedeva G.A., Ermachenko A.V. Psoriasis epidemiology issues. Klinicheskaya Dermatologiya i Venerologiya = Russian Journal of Dermatology and Venereology. 2015;1:12-16. DOI 10.17116/klinderma2015112-16. (in Russian)

Korotaeva T.V. Prospects for using interleukin-17 inhibitors, a new class of drugs for targeted therapy of psoriatic arthritis. Nauchno-Prakticheskaya Revmatologiya = Rheumatology Science and Practice. 2016;54(3):346-351. DOI 10.14412/1995-4484-2016-346-351. (in Russian)

Ma W.Y., Jia K., Zhang Y. IL-17 promotes keratinocyte proliferation via the downregulation of C/EBPα. Exp. Ther. Med. 2016;11(2):631-636. DOI 10.3892/etm.2015.2939.

Manczinger M., Kemény L. Novel factors in the pathogenesis of psoriasis and potential drug candidates are found with systems biology approach. PLoS One. 2013;8(11):e80751. DOI 10.1371/journal.pone.0080751.

Matsushime H., Ewen M.E., Strom D.K., Kato J.Y., Hanks S.K., Roussel M.F., Sherr C.J. Identification and properties of an atypical catalytic subunit (p34PSK-J3/cdk4) for mammalian D type G1 cyclins. Cell. 1992;71(2):323-334.

Mezentsev A., Nikolaev A., Bruskin S. Matrix metalloproteinases and their role in psoriasis. Gene. 2014;540(1):1-10. DOI 10.1016/j.gene. 2014.01.068.

Michalek I.M., Loring B., John S.M. A systematic review of worldwide epidemiology of psoriasis. J. Eur. Acad. Dermatol. Venerol. 2017; 31(2):205-212. DOI 10.1111/jdv.13854.

Mills K.H. Induction, function and regulation of IL-17-producing T cells. Eur. J. Immunol. 2008;38(10):2636-2649. DOI 10.1002/eji. 200838535.

Mishina O.S., Dvornikov A.S., Dontsova E.V. Psoriasis and psoriatic arthritis: Analysis of 2009–2011 incidence rates in the Russian Federation. Doctor.ru. 2013;4:52-55. (in Russian)

Mogulevtseva J.A., Mezentsev A.V. Optimization of lentiviral transduction of immortalized epidermal keratinocytes. Modern Science: Theory and Practice. 2017;13:123-134. (in Russian)

Nair R.P., Duffin K.C., Helms C., Ding J., Stuart P.E., Goldgar D., Gudjonsson J.E., Li Y., Tejasvi T., Feng B.J., Ruether A., Schreiber S., Weichenthal M., Gladman D., Rahman P., Schrodi S.J., Prahalad S., Guthery S.L., Fischer J., Liao W., Kwok P.Y., Menter A., Lathrop G.M., Wise C.A., Begovich A.B., Voorhees J.J., Elder J.T., Krueger G.G., Bowcock A.M., Abecasis G.R., for the Collaborative Association Study of Psoriasis. Genome-wide scan reveals association of psoriasis with IL-23 and NF-κB pathways. Nat. Genet. 2009; 41(2):199-204. DOI 10.1038/ng.311.

NCBI Probe. 2015. Available at https://www.ncbi.nlm.nih.gov/probe/

Noh M., Yeo H., Ko J., Kim H.K., Lee C.H. MAP17 is associated with the T-helper cell cytokine-induced down-regulation of filaggrin transcription in human keratinocytes. Exp. Dermatol. 2010;19(4):355-362. DOI 10.1111/j.1600-0625.2009.00902.x.

Pagano M., Pepperkok R., Verde F., Ansorge W., Draetta G. Cyclin A is required at two points in the human cell cycle. EMBO J. 1992; 11(3):961-971.

Peric M., Koglin S., Dombrowski Y., Gross K., Bradac E., Büchau A., Steinmeyer A., Zügel U., Ruzicka T., Schauber J. Vitamin D analogs differentially control antimicrobial peptide/“alarmin” expression in psoriasis. PLoS One. 2009;4(7):e6340. DOI 10.1371/journal.pone.0006340.

Peric M., Koglin S., Kim S.M., Morizane S., Besch R., Prinz J.C., Ruzicka T., Gallo R.L., Schauber J. IL-17A enhances vitamin D3-induced expression of cathelicidin antimicrobial peptide in human keratinocytes. J. Immunol. 2008;181(12):8504-8512.

Rao K.S., Babu K.K., Gupta P.D. Keratins and skin disorders. Cell. Biol. Int. 1996;20(4):261-274.

Reischl J., Schwenke S., Beekman J.M., Mrowietz U., Stürzebecher S., Heubach J.F. Increased expression of Wnt5a in psoriatic plaques. J. Invest. Dermatol. 2007;127(1):163-169. DOI 10.1038/sj.jid.5700488.

Rukavishnikova V.M. Change of nails at psoriasis. Vestnik Dermatologii i Venerologii = Bulletin of Dermatology and Venereology. 2009;2:71-79. (in Russian)

Schindelin J., Rueden C.T., Hiner M.C., Eliceiri K.W. The ImageJ ecosystem: An open platform for biomedical image analysis. Mol. Reprod. Dev. 2015;82(7-8):518-529. DOI 10.1002/mrd.22489.

Scott K.A., Arnott C.H., Robinson S.C., Moore R.J., Thompson R.G., Marshall J.F., Balkwill F.R. TNF-α regulates epithelial expression of MMP-9 and integrin αvβ6 during tumour promotion. A role for TNF-α in keratinocyte migration? Oncogene. 2004;23(41):6954-6966. DOI 10.1038/sj.onc.1207915.

Seo K.Y., Kitamura K., Han S.J., Kelsall B. Th17 cells mediate inflammation in a novel model of spontaneous experimental autoimmune lacrimal keratoconjunctivitis with neural damage. J. Allergy Clin. Immunol. 2017; pii: S0091-6749(17)31504-X. DOI 10.1016/j.jaci. 2017.07.052.

Seo M.D., Kang T.J., Lee C.H., Lee A.Y., Noh M. HaCaT keratinocytes and primary epidermal keratinocytes have different transcriptional profiles of cornified envelope-associated genes to T helper cell cytokines. Biomol. Ther. (Seoul). 2012;20(2):171-176. DOI 10.4062/biomolther.2012.20.2.171.

Shilova L.N., Pan’shina N.N., Chernov A.S., Trubenko Y.A., Khortieva S.S., Morozova T.A., Pan’shin N.G. Immunopathological significance of interleukin-17 in psoriatic arthritis. Sovremennye Problemy Nauki i Obrazovaniya = Current Problems of Science and Education. 2015;6:54. (in Russian)

Sobolev V.V., Starodubtseva N.L., Soboleva A.G., Rakhimova O.Yu., Korsunskaya I.M., Piruzian E.S., Minnibaev M.T., Krivoschapov L., Bruskin S.A., Voron’ko O.E. Role of interleukins in psoriasis. Sovremennye Problemy Dermatovenerologii, Immunologii i Vrachebnoy Kosmetologii = Current Problems of Dermatovenerology, Immunology, and Medical Cosmetology. 2010;5(5):79-84. (in Russian)

Soboleva A.G., Mezentsev A., Zolotorenko A., Bruskin S., Pirusian E. Three-dimensional skin models of psoriasis. Cells Tissues Organs. 2014a;199(5-6):301-310. DOI 10.1159/000369925.

Soboleva A.G., Zolotarenko A.D., Sobolev V.V., Bruskin S.A., Piruzian E.S., Mezentsev A.V. Genetically predetermined limitation in HaCaT cells that affects their ability to serve as an experimental model of psoriasis. Russ. J. Genetics (Moscow). 2014b;50(10):1081-1089. DOI 10.1134/S1022795414100123.

Starodubtseva N.L., Sobolev V.V., Soboleva A.G., Nikolaev A.A., Bruskin S.A. Genes expression of metalloproteinases (*MMP-1*, *MMP-2*, *MMP-9*, and *MMP-12*) associated with psoriasis. Russ. J. Genetics (Moscow). 2011;47(9):1117-1123. DOI 10.1134/S102279541109016X.

Tohyama M., Hanakawa Y., Shirakata Y., Dai X., Yang L., Hirakawa S., Tokumaru S., Okazaki H., Sayama K., Hashimoto K. IL-17 and IL-22 mediate IL-20 subfamily cytokine production in cultured keratinocytes via increased IL-22 receptor expression. Eur.

J. Immunol. 2009;39(10):27792788. DOI 10.1002/eji.200939473.

Vegfors J., Ekman A.K., Stoll S.W., Bivik Eding C., Enerbäck C. Psoriasin (S100A7) promotes stress-induced angiogenesis. Br. J. Dermatol. 2016;175(6):1263-1273. DOI 10.1111/bjd.14718.

Wells J.M., Gaggar A., Blalock J.E. MMP generated matrikines. Matrix Biol. 2015;44-46:122-129. DOI 10.1016/j.matbio.2015.01.016.

Wu Y., Zhu L., Liu L., Zhang J., Peng B. Interleukin-17A stimulates migration of periodontal ligament fibroblasts via p38 MAPK/NF-κB-dependent MMP-1 expression. J. Cell. Physiol. 2014;229(3):292-299. DOI 10.1002/jcp.24444.

Yazici A.C., Tursen U., Apa D.D., Ikizoglu G., Api H., Baz K., Tasdelen B. The changes in expression of ICAM-3, Ki-67, PCNA, and CD31 in psoriatic lesions before and after methotrexate treatment. Arch. Dermatol. Res. 2005;297(6):249-255. DOI 10.1007/s00403-005-0602-8.

Zolotarenko A., Chekalin E., Mesentsev A., Kiseleva L., Gribanova E., Mehta R., Baranova A., Tatarinova T.V., Piruzian E.S., Bruskin S. Integrated computational approach to the analysis of RNA-seq data reveals new transcriptional regulators of psoriasis. Exp. Mol. Med. 2016;48(11):e268. DOI 10.1038/emm.2016.97.