Influence of *trim14* overexpression on the transcriptional changes in embryonic cells

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Running title: Transcriptional changes in *trim14*-overexpressed embryonic cells

Keywords: mouse embryonic stem cells; *trim14*; differentiation; embryoid bodies; HEK293T

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Abstract

TRIM14 is known as a representative of the tripartite motif (TRIM) family of proteins. The *trim* genes are implicated in a variety of human pathologies, from Mendelian inherited disoders to cancer, and are involved in cellular response to viral infection. Previously we obtained mouse embryonic stem cells (MESC) overexpressing the human gene *trim14* (ES-hPub) and showed that *trim14* did not affect MESC proliferation but it contributed to MESC mesodermal differentiation and inhibited MESC ectodermal one. To elucidate the molecular mechanisms underlying the TRIM14 functions, we examined the transcriptome of ES-hPub by subtractive hybridization and real-time PCR and found a number of genes which expression enhanced by *trim14* in embryonic cells: *hsp90ab1*, *prr13*, *pu.1*, *tnfrsf13c (baff-r)*, *tnfrsf13b (taci), hlx1, hbp1, junb, pdgfrb*. The further analysis of the MESC at the initial stage of differentiation (embryoid bodies formation) showed the essential changes in the revealed genes expression. Overexpression of *trim14* in the human embryonic kidney cells

(HEK293T) also induced the enhanced expression of several found genes (*hsp90ab1*, *prr13*, *pu.1*, *tnfrsf13c (baff-r)*, *tnfrsf13b (taci), hlx1*). These genes are reported to be involved in the mesodermal differentiation at different stages. In sum, we found the genes that participated with the *trim14* in the mesoderm-directed differentiation of MESC. These results provide a first insight into the *trim14*-directed intracellular signaling pathway that promotes mesodermal differentiation in embryonic cells.

Keywords: mouse embryonic stem cells; *trim14*; differentiation; embryoid bodies; HEK293T

Abbreviations: TRIM, tripartite motif; MESC, mouse embryonic stem cells; ES-hPub, mouse embryonic stem cells overexpressing the human gene *trim14*; ES-DNA3, mouse embryonic stem cells transfected with the pcDNA3 control plasmid; HEK293T, human embryonic kidney cells; HIV, human immunodeficiency virus; SIV, simian immunodeficiency virus; EB, embryoid bodies.

1. Introduction

Trim14 (*pub*, *KIAA0129*) encodes the protein TRIM14. This protein belongs to the family of TRIM proteins with a conserved domain architecture (known as RBCC) that is characterized by RING (R) finger domain, one or two B-box domains (B1 and B2), a Coiled-coil domain and a variable C-terminus (Reymond et al., 2001). According to these days' researches, about 100 genes encoding TRIM proteins have been found in the human genome. As shown recently, TRIM proteins are implicated in a variety of cellular functions, including differentiation, apoptosis and immunity. An increasing number of TRIM proteins are known to be involved in processes associated with antiviral activities or innate immunity (Torok and Etkin, 2000; Nisole et al., 2005; Ozato et al., 2008; Munir, 2010). However, we still know almost nothing about the biological and molecular mechanisms mediated by *trim14* gene. It was found that the mouse homolog of the TRIM14 protein Pub interacts with transcription factor Spi.1 (PU.1). Pub inhibited the transcriptional activity of PU.1 in hemocytes and could be essential for differentiation and proliferation of myeloid and lymphoid cells (Hirose et al., 2003). Earlier we have found that the expression of *trim14* was considerably enhanced in HIV-associated human (Nenasheva et al., 2001) and SIV-associated monkey (Tarantul et al., 2000) lymphomas.

In order to study the functions of *trim14* we obtained MESC where the human gene *trim14* showed enhanced expression (cell line ES-hPub) (Novosadova et al., 2005). Further we demonstrated that *trim14* gene did not affect proliferation of these MESC (Novosadova et al., 2012) but it contributed to a mesodermal differentiation of the same cells and inhibited their ectodermal differentiation (Novosadova et al., 2009). Moreover, we showed that the embryoid bodies (EB) were

formed one day earlier in cell line ES-hPub than in control ES-DNA3 cell line (MESC were transfected with the pcDNA3 control plasmid) (Novosadova et al., 2012).

In the present investigation, we revealed the genes upregulated in ES-hPub cell line by using subtractive hybridization and real-time PCR. We also have studied the changes in the gene expression in cells ES-hPub at initial stage of their differentiation (formation of EB) and in human embryonic kidney cells HEK293T (these are the cells at later stage of differentiation) transfected with the *trim14*. As a result we have detected the several genes connected with an enhanced expression of the *trim14* in the different types of cells (*hsp90ab1*, *prr13*, *pu.1*, *tnfrsf13c (baff-r)*, *tnfrsf13b (taci), hlx1*). We suppose that these genes are involved in MESC differentiation in mesodermal direction caused by *trim14*.

2. Materials and methods

2.1. Cells.

MESC line ES-DNA3 was obtained by transfection with pcDNA3 vector (with the neomycin resistance) (Promega, USA), MESC line ES-hPub was obtained by transfection with plasmid pcDNA3-pub (a full-size cDNA insert of the human gene *trim14* (*pub, KIAA0129)* (NM_014788.2) was cloned into *HindIII/NotI* sites of pcDNA3) during our previous study (Novosadova et al., 2005). MESC lines (ES-DNA3 and ES-hPub) were cultured as described in Novosadova et al. (2009). Induction of the differentiation with EB formation was performed as described in Novosadova et al. (2009).

The line of human embryonic kidney cells (HEK293T) was received from the collection of D. I. Ivanovsky Institute of Virology. The cells were grown at 37^0C and 5% CO_2 in the DMEM with 10% bovine fetal serum (BFS) (Gibco, USA).

2.2. Vector construction.

The resulting plasmid pcDNA4-pub contained a full-size insert of cDNA from the human gene *trim14* (*pub, KIAA0129;* NM_014788.2) into *Hind*III/*Not*I sites of pcDNA4 vector (Promega, USA) with the zeocin resistance. We constructed the plasmid pcDNA4-pub for the HEK293T cells transfection based on the pcDNA4 because the HEK293T cells were resistant to neomycin.

2.3. Transfection of HEK293T cells.

The HEK293T cells were transfected with vectors pcDNA4 (Promega, USA) (control vector) and pcDNA4-pub using Unifectin-56™ (Unifect, The transfection group, Russia) by standard procedure (respectively, HEK293T and HEK-*trim14* cell lines). 12 h after transfection selective

antibiotic zeocin was added (200 μg/ml). Efficiency of transfection was estimated with real-time PCR as described below. PCR primers for human *trim14* are shown in Table 1.

Table 1.

Nucleotide sequences of the primers used to amplify the human genes examined.

Human genes	Primer sequences (5'→3')
act	TGCTTCTAGGCGGACTATGAC AGAAGTGGGGTGGCTTTTAGG
odc	TGAGACCTTCGTGCAGGCAATCTCT CGATCGAGGCCATCACATGTTGGTC
pub	GCAGCAGCACATTGACAACA TCCACGAGGCCCTTAAAGAA
pu.1	TGTCAAGGGAGGGGGCTCACC GGCACCAGGTCTTCTGATGGCT
prim2	AAGCCTTGCGGGAAAATCACCATC GGGCACCCATGATAATCCCCTTGG
taci	GCTGGGGCTCTGCCTGTGTG GCACCCCCACCTTCCAGCAC
hlx1	GTCGCGCGCTGTGTTCTCCA CTCAGTCCGCTCCGTGTCGC
tipin	CAGACACATGGAGCACTGGGCA AACCGTGTGTGCCCTGGGTG
hsp90ab1	TGGAGCGAGTGCGGAAACGG GGCTGTCCAGCCGTAGGTGC
hbp1	ATGGCGACGGGTTTGTCAGAG GGTGGAGGGCGTGCATAGGA
ganab	GGGCCCCTAGGGTCTCGCAA GGCGATATGGCTCCCCACCCT
prr13	TAGGCAGTAGCAGAGGCGGGTG GTGGGTGGGCAGGATTGGAACC
baffr	CACTGGTCCTGGCGCTGGTC CTCCTGCCGGCTCCCTGCTA
junb	ACCGGGAGCTCGTACCCGAC ACAGCCGTTGCTGACGTGGG
pdgfrb	GTCAGCTGGGCTCCTGGGAGAT TGGTGGGTGAGGGGCAAACTC

2.4. Isolation of total RNA for subtractive hybridization.

Total RNA was prepared with the reagent Trizol (Invitrogen) as recommended by manufacturer. Reverse transcription was performed using the Mint kit for reverse transcription (Evrogen Ltd, Russia) following the manufacturer's instructions.

2.5. Subtractive hybridization.

Subtractive hybridization carried out using the PCR-Select™ cDNA Subtraction Kit (Clontech). Driver cDNA was synthesized on mRNA from cell line ES-DNA3, and tracer cDNA was synthesized on mRNA from the cells ES-hPub. Three hybridization cycles were run as recommended by manufacturer. The clones from the obtained subtractive libraries were selected and analyzed using the two-step differential screening (Nenasheva et al., 2005).

2.6. Pseudo-Northern-blot hybridization.

Tracer and driver cDNAs were separated by electrophoresis in 1% agarose gel and then were transferred onto the Hybond-N filter using the standard capillary method (Sambrook et al., 1989). The filters were hybridized with the probes labeled using the Prime-a Gene Labeling System (Promega), with [α^{32}P] dATP (Obninsk, Russia). The probes were the labeled cDNA samples obtained by subtractive hybridization. A labeled PCR fragment of the mouse β-actin gene was used as a gel loading control (primer sequences are shown in Table 2). The blots were quantified using ImageQuant (Amersham, USA).

2.7. Quantitative real-time PCR.

Total RNA was isolated from the cell lines (ES-DNA3 and ES-hPub, HEK293T and HEK-*trim14*) using the reagent Trizol (Invitrogen, USA) as recommended by manufacturer. The reverse transcription reaction was performed using the M-MLV Reverse Transcriptase (Promega, USA). The obtained cDNA was amplified using CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad). Reaction conditions were the following: denaturation at 95 °C (3 min), then 40 cycles (95 °C, 15 s, 60 °C, 20 s, 72 °C, 45 s). The reaction mixture qPCRmix-HS SYBR (Evrogen Ltd, Russia) was used. Primer sequences are shown in Tables 1 and 2.

2.8. Statistics.

Qantitative PCR data are expressed as mean±SEM. The significant difference was calculated with Student's t-test and the one-way analysis of variance (ANOVA). A P value of $p<0.05$ was considered to be significant.

Table 2.

Nucleotide sequences of the primers used to amplify the mouse genes examined.

Mouse genes	Primer sequences (5'-3')	Mouse genes	
act	TCATGAAGTGTGACGTTGACATCCGTAAAG CCTAGAAGCATTTGCGGTGCACGATGGAGG	*rhob*	TGGACAGCCCGGACTCTCT TGCGCAGGTCTTTTTTGTTG (Stedl et al., 2006)
odc	CAGTTGGTGCAGGGGCTTGGG CACAGCGGGCATCCGACACTG	*socs2*	CCAGAAGCCCCACGGAAT TTGTTAATGGCGAGTCGACAGA (Stedl et al., 2006)
pub	CCCATTTGGAAGACGCCG AGGGTGGCTCAGCTCCG	*tek*	TTTGTTTGGCCATGTGACAATT CCAAAGGTTCTTAGTCTCCTAGCAA (Stedl et al., 2006)
pu.1	AGAAGCTGATGGCTTGGAGC GCGAATCTTTTTCTTGCTGCC (Stedl et al., 2006)	*hlx*	AGTCCTCAAAGCCCGGAGAT GCACAAAGTCCAGCCAGTGTAG (Stedl et al., 2006)
c-*jun*	GTCCCCTATCGACATGGAGTCT GGAGTTTTGCGCTTTCAAGGT (Stedl et al., 2006)	*pdgfrb*	AGGAAGCACCCACTCCTTTCA CCTATCACAGGCGTGGACAA (Stedl et al., 2006)
junb	CTGTGTCCCCCATCAACATG TTCCGCTTCCGGCACTT (Stedl et al., 2006)	*wnt5b*	AGCACCGTGGACAACACATC TGCATACGTGAAGGCAGTCTCT (Stedl et al., 2006)
baffr	AGATGGGCATGGTGGTACACA TGGAACTTGCTATGTAGACCAGGAT (Stedl et al., 2006)	*rab19*	CACACGGCGGTCCACATT CTGATTTGTTCCCGATCAGCAT (Stedl et al., 2006)
dnmt2	CCTACGAAGATATGCCCATGAGT CACAGGTCTCTGAACCAGATAAAAGA (Stedl et al., 2006)	*tipin*	GAAGCCTAACAGAAGAGCAGCAACA CCCAAGCTTGCAGGATTCTGTTGTA
pak1	CTCTGCAAGTGGACATGCTTACTC CCTACCACAGCAGGAGAACCA (Stedl et al., 2006)	*hsp90ab1*	GCTGGACAGCCAACATGGAACG AGGGCAGGGCTTCTTCCAGGAG
taci	TGTCTGGGAGACGAACCTATTTC GGTTCCAGAATCGTCTTGCATT (Stedl et al., 2006)	*hbp1*	AGCTCAGGGACTGTGAGTGCCA GGTGAGACCAAGGTCTTCCAAGC
egr1	GAAACAGCCATGTCCAAGTTCTT AGGGCCAGGCATGTGATG (Stedl et al., 2006)	*ganab*	ATGGATGCGTGTGAGGCGCT CCAGTCGGATGCCACGCTGA
evi5	CACCCTAAGACTTGAAGGATGTTCTA TGTGTCACCTGGGCAGAAAC (Stedl et al., 2006)	*prr13*	CCCTCCGTGTCGTCCCCCTT AGCCCTTGACAGCCGGGACA
fosb	AGAAGACCCCGAGAAGAGACACT CGACGGTTCCTGCACTTAGC (Stedl et al., 2006)	*prim2*	ACCCACCAGGCCAAGGGGATT TCTAGGGAGGCCAGCGCAGAT

3. Results.

3.1. The upregulated genes in the MECS overexpressed trim14.

To find genes that participate with the *trim14* in the processes of stem cells differentiation we performed the subtractive hybridization between cDNAs synthesized on mRNAs from transfected MESC lines: ES-hPub with enhanced expression of the *trim14* (tracer) and ES-DNA3 (transfected with the control plasmid pcDNA3) (driver). The two-step differential screening of subtracted 104 clones yielded 15 clones with differential signals (data not shown). Pseudo-Northern-blotting was applied to confirm the results of subtractive hybridization and differential screening (Fig. 1).

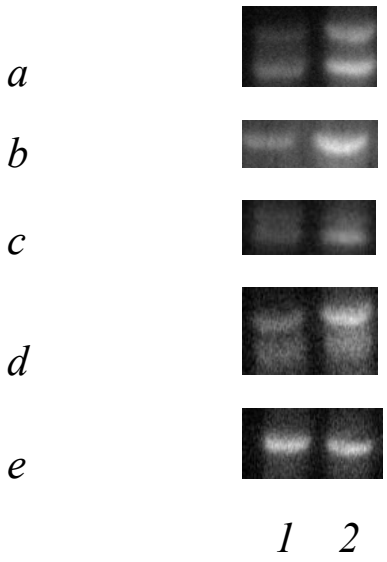


Fig. 1. Pseudo-Northern-blot hybridization of cDNAs synthesized on mRNAs from the control cell line ES-DNA3 (lane 1), and from cell line ES-hPub (lane 2). Probes in *a, b, c, d* were the labeled cDNA inserts from the clones 52, 91, 95, 100, respectively, selected after subtraction hybridization and differential screening (See Table 3); *e* – labeled PCR fragment of the β-actin gene used as a control for gel loading.

The obtained clones were sequenced, and their nucleotide sequences were compared with the database (www.ncbi.nlm.nih.gov) (Table 3). For the further analysis, we chose the genes that corresponded to clones revealed the strongest differential signals during both differential screening and pseudo- Northern-blotting.

Table 3.

Mouse genes corresponding to the sequences of clones selected by subtractive hybridization and differential screening.

Clone	Gene	ID
11	microfibrillar-associated protein 3 (Mfap3), transcript variant 1	NM_145426.2
13	coiled-coil domain containing 99 (Ccdc99)	NM_027411.2
25	selenophosphate synthetase 2 (Sephs2)	NM_009266.3
37	ring finger protein 181 (Rnf181)	NM_025607.3
52	heat shock protein 90 alpha (cytosolic), class B member 1 (Hsp90ab1)	NM_008302.3
64	3-hydroxy-3-methylglutaryl-Coenzyme A lyase (Hmgcl)	NM_008254.2
87	COP9 (constitutive photomorphogenic) homolog, subunit 4 (Arabidopsis thaliana) (Cops4)	NM_012001.2
89	AT rich interactive domain 2 (ARID, RFX-like) (Arid2)	NM_175251.3
91	alpha glucosidase 2 alpha neutral subunit (Ganab)	NM_008060.1
92	family with sequence similarity 120, member A (Fam120a)	NM_001033268.2
93	proline rich 13 (Prr13), transcript variant 2	NM_001170911.1
94	FK506 binding protein 3 (Fkbp3)	NM_013902.4
95	DNA primase, p58 subunit, Prim2	NM_008922.1
100	timeless interacting protein (Tipin)	NM_025372.3
102	high mobility group box transcription factor 1 (Hbp1), transcript variant 1	NM_153198.2

3.2. The expression of the subtracted genes in ES-hPub cells changed at the stage of EB formation.

Previously we showed that overexpression of *trim14* affected the period of formation and the number of EB compared with the control (Novosadova et al., 2005). In the present study, we used quantitative real-time PCR to analyze the expression of the selected genes in ES-hPub cells before and during the stage of EB formation in comparison with the control cells ES-DNA3.

It was found out that significantly overexpressed in ES-hPub gene *prr13* demonstrated sharp decrease in expression at EB formation. The genes *hbp1*, *ganab, prim2*, *tipin* which expression in ES-hPub changed slightly showed significant increase in expression during EB formation compared with the control cells. On the contrary, increase in *hsp90ab1* expression in ES-hPub was noticeable only before formation of the EB (Fig. 2).

To summarize, the pattern of gene expression observed in ES-hPub underwent considerable changes in the process of EB formation.

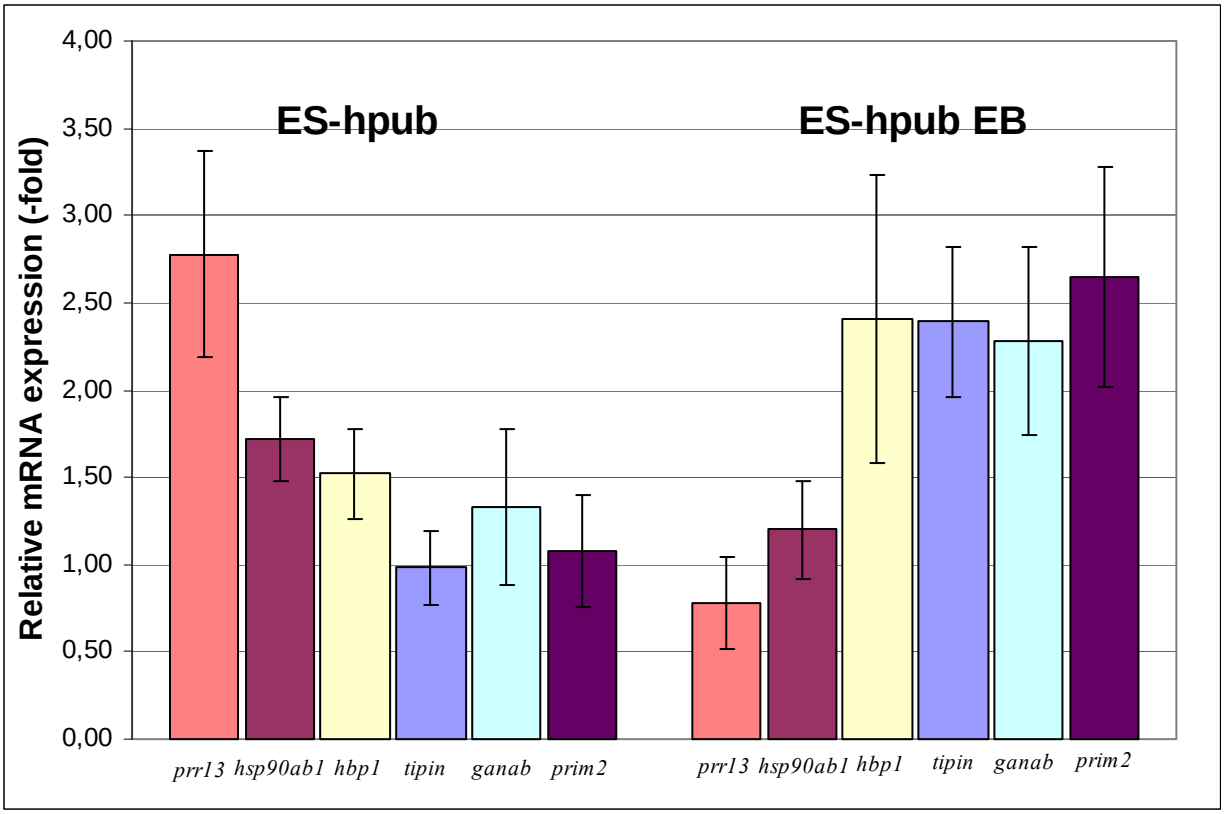


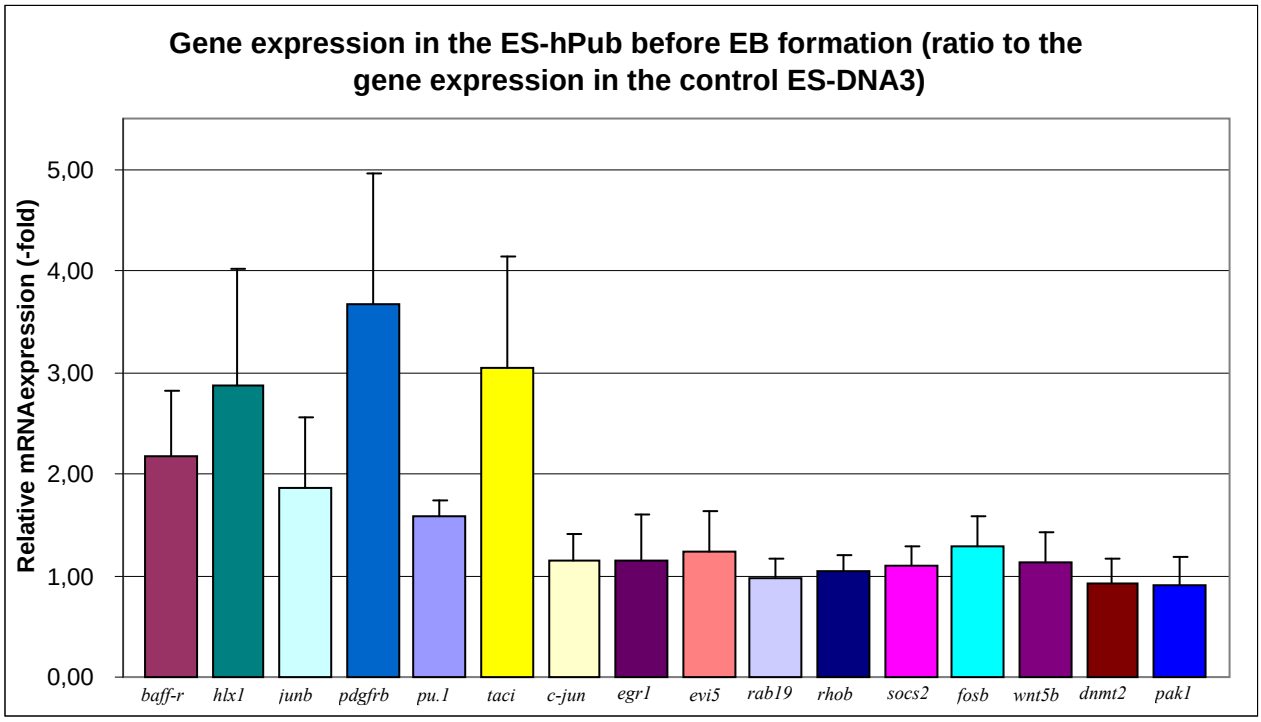
Fig. 2. Changes in the mRNA expression of the selected genes in ES-hPub cells before and during the EB formation relative to control (ES-DNA3 cells) estimated by quantitative real-time PCR. The gene levels were normalized to two mouse genes: β-actin and ornitin decarboxylase (*odc*) (the average value for each sample was taken) (n=4-7; $p<0.05$).

3.3. The expression of the genes connected with pu.1 (Steidl et al., 2006) increased in cell line ES-hPub before and during EB formation.

As reported by Hirose et al. (2003), mouse homolog of the protein Trim14 (PUB) bound to transcription factor PU.1 and inhibited its activity. Consequently, the genes connected with PU.1 were of our special attention.

Steidl et al. (2006) analyzed the expression of various genes after *pu.1* knockdown in the mouse hemopoietic stem cells (HSC) and showed that mRNA expression of several genes (*tnfrsf13c (baff-r), junb, c-jun, dnmt2, pak1, tnfrsf13b (taci), egr1, evi5, fosb, rhob, socs2, tek*) decreased and the expression of *wnt5b, hlx1, rab19, pdgfrb* enhanced in these cells in comparison with the control HSC. According to these data, we suggested that *trim14* overexpression and, consequently, increased amount of the protein TRIM14 in the cells, would lead to decrease in PU.1 transcriptional activity. Consequently, we hypothesized that the gene expression patterns would be similar in cells with overexpression of *trim14* and in cells with knockdown of *pu.1*. However, the results of our investigation did not coincide with our assumption (Fig. 3a). So, small but statistically significant increase in mRNA expression was found for *pu.1* in the cells ES-hPub before EB formation. The

a



b

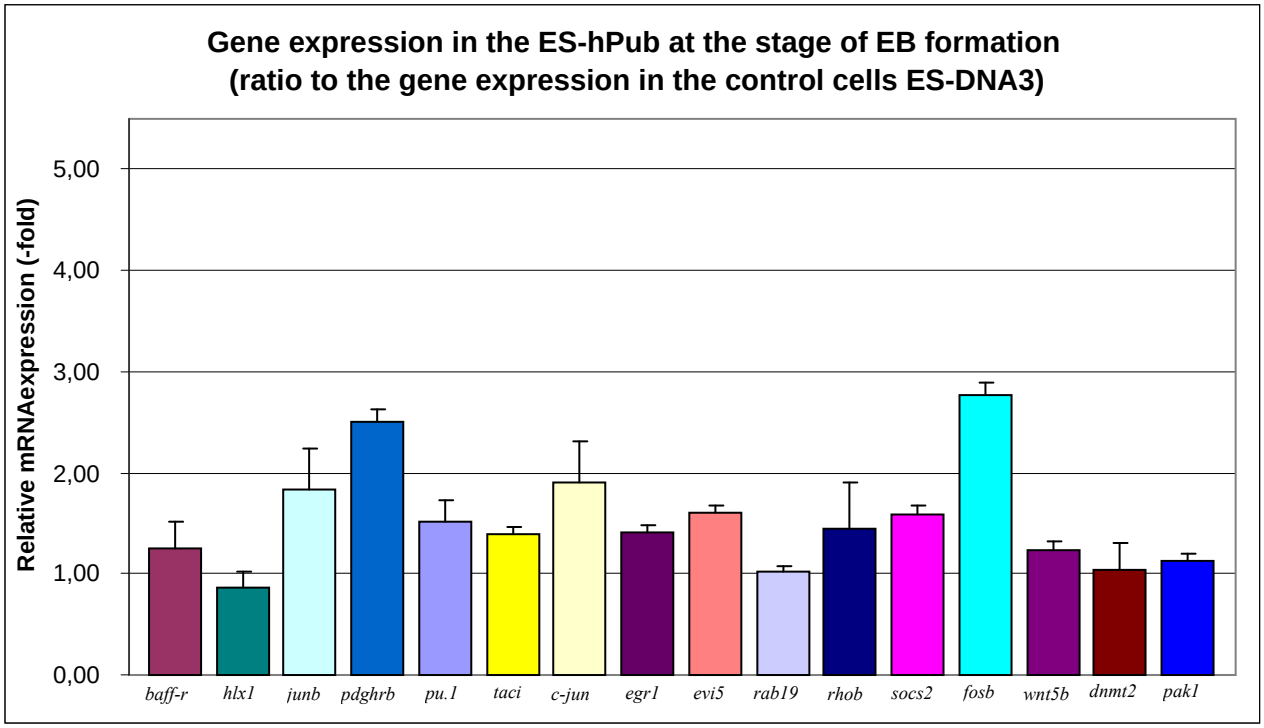


Fig. 3. Changes in the expression of the mouse genes at *pu.1* knockdown (Steidl et al., 2006) were estimated by means of real-time PCR. The mRNA expression levels in cell line ES-hPub (a) before the stage of EB formation and (b) at the stage of EB formation are shown in relation to the expression levels in control cell line ES-DNA3. The gene levels were normalized to two mouse

genes: β-actin and ornitin decarboxylase (*odc*) (the average value for each sample was taken) (n=4-14; $p<0.05$).

genes *tnfrsf13c (baff-r), junb, tnfrsf13b (taci)* demonstrated enhanced expression in ES-hPub, in parallel with the *pu.1* mRNA expression (they revealed the decreased expression at *pu.1* knockdown as reported by Steidl et al., 2006). On the contrary, Steidl et el. (2006) reported the enhanced expression of *hlx1* and *pdgfrb* after knockdown of the gene *pu.1,* but these genes also showed enhanced expression in ES-hPub cells compared with the control. Expression of other genes remained unchanged (Fig 3a).

The expression pattern of the above-mentioned genes changed considerably at EB formation in the cells ES-hPub. Expression level of the genes *c-jun, egr1, evi5, fosb, rhob, socs2*, increased, while transcription level of the genes *tnfrsf13c (baff-r), tnfrsf13b (taci)* considerably decreased, and expression level of the gene *hlx1* fell below the control. mRNA levels of *pdgfrb* and *pu.1* also somewhat decreased compared with the control. The only one gene, *junb*, showed the same enhanced expression before and during EB formation (Fig. 3b).

3.4. The several upregulated genes in ES-hPub were affected in HEK293T cells with trim14 overexpression.

We now asked whether the genes connected with *trim14* in MESC could participate in the processes mediated by *trim14* in other types of cells. Therefore, we obtained the human embryonic kidney cells HEK293T with *trim14* overexpression (HEK-*trim14*) (Fig. 4).

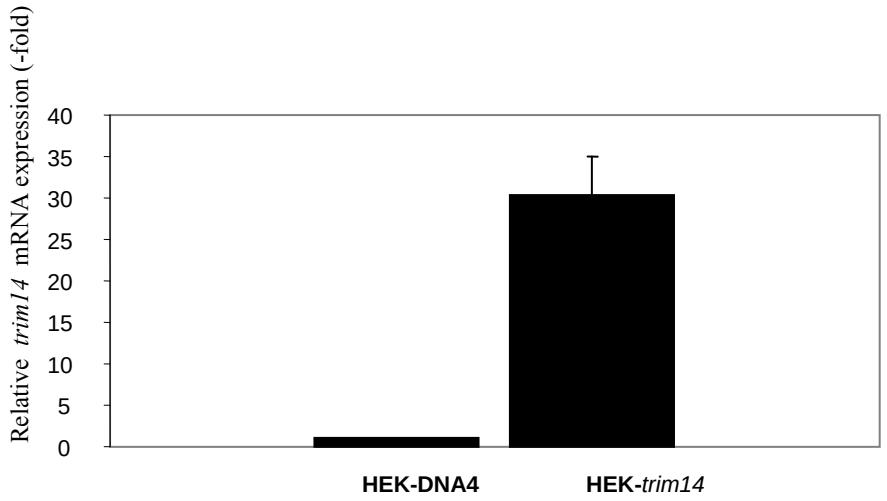
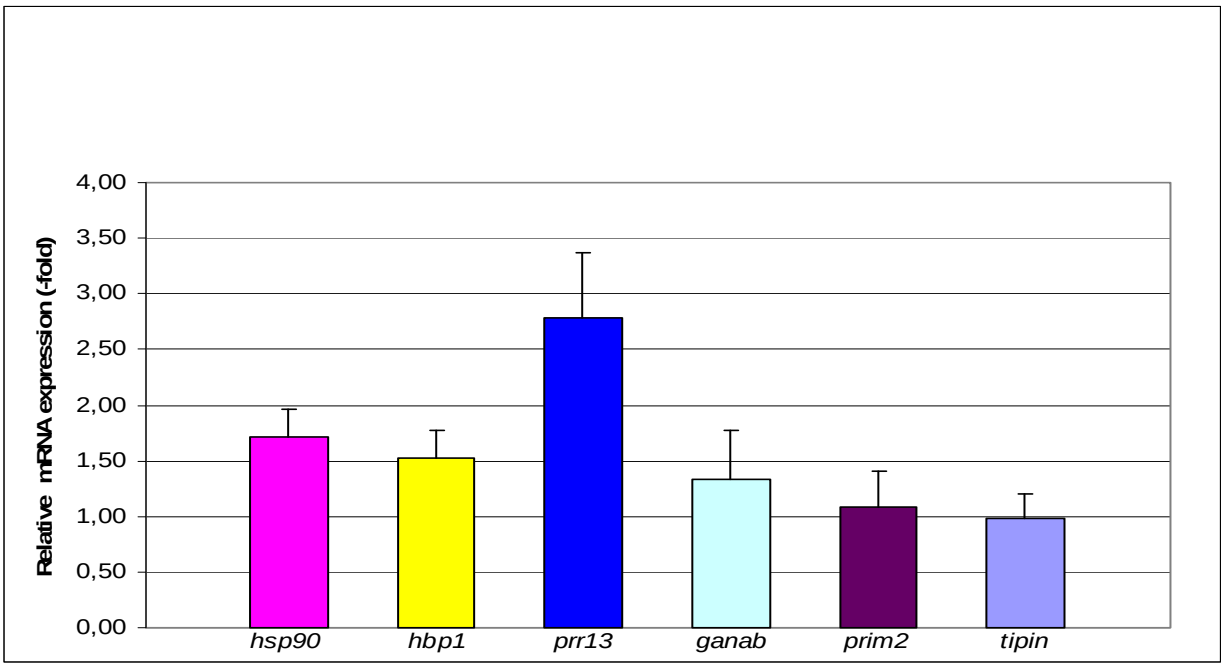


Fig. 4. Analysis of *trim14* mRNA expression in cell lines HEK-*trim14* and HEK-DNA4 (control) by quantitative real-time PCR (n=3; $p<0.01$). The gene levels were normalized to two mouse genes: β-actin and ornitin decarboxylase (*odc*) (the average value for each sample was taken).

a



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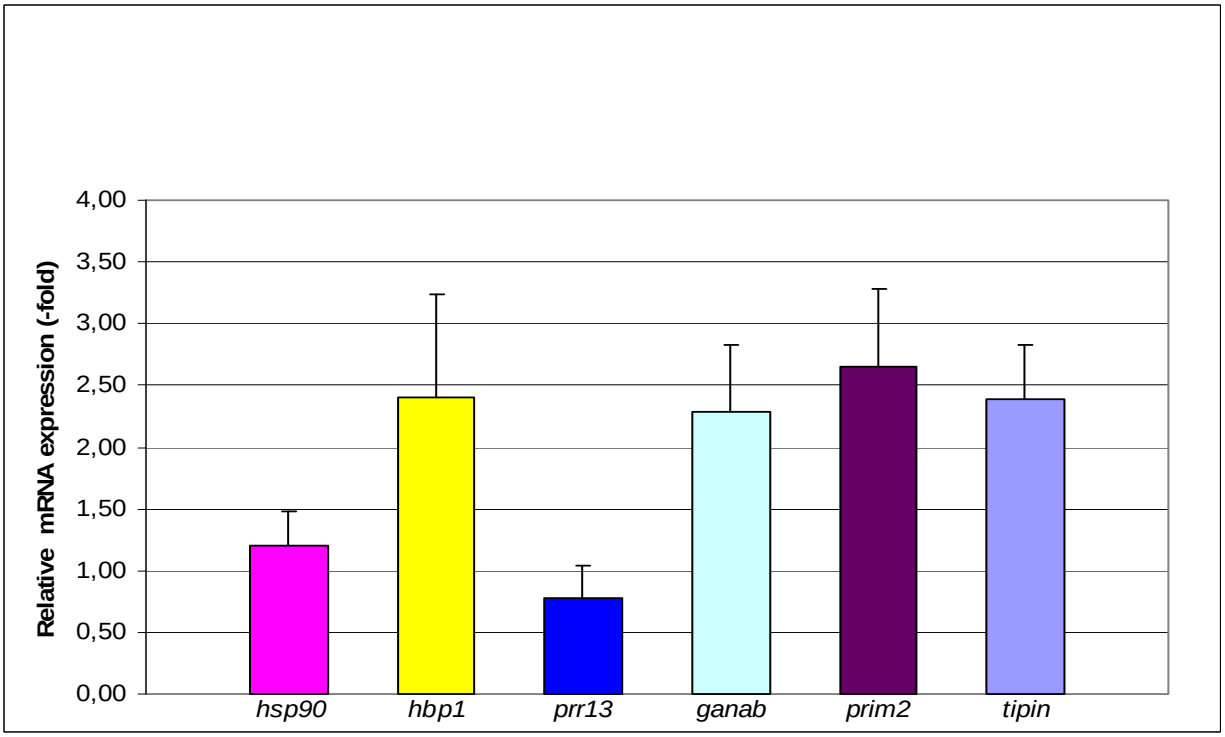

Fig. 5. The gene expression changes in HEK-*trim14* compared to the control HEK-DNA4 cells. The mRNA relative expression levels of human genes in cell line HEK-*trim14* (ratio to HEK-DNA4) corresponding to those (a) detected by subtractive hybridization in ES-hPub or (b) connected with *pu.1* (Steidl et al., 2006) analyzed by quantitative real-time PCR. The gene levels were normalized to two mouse genes: β-actin and ornitin decarboxylase (*odc*) (the average value for each sample was taken) (n=3; $p<0.05$).

Expression analysis of the human genes corresponding to those affected in ES-hPub in HEK-*trim14* compared with the control cells HEK-DNA4 was shown at Fig. 5ab. Among the genes, *hsp90ab1, prr13, pu.1*, *tnfrsf13c (baff-r), tnfrsf13b (taci)* and *hlx1* showed statistically significant increase in expression in HEK-*trim14*. The genes *hbp1*, *ganab, prim2*, and *tipin* which expression enhanced in ES-hPub during EB formation expressed at the control level.

4. Discussion.

It is known that the TRIM proteins are involved in diverse cellular functions, including cell proliferation, differentiation, apoptosis, and morphogenesis (Kawai and Akira, 2011; McNab et al., 2011). Previously we have revealed the *trim14* mRNA upregulation in HIV-associated human lymphomas (Nenasheva et al., 2005) and SIV-associated monkey lymphomas (Tarantul et al., 2000).

In order to investigate the functions of *trim14* we obtained MESC with the human *trim14* gene enhanced expression (ES-hPub) (Novosadova et al., 2005) and showed that *trim14* did not affect MESC proliferation (Novosadova et al., 2012). On the other hand, it led to MESC mesodermal differentiation: enhanced expression of the lymphoid-specific genes (*c-kit, IL-7*) and increase of a number of the contracting cardiomyocytes were observed. Besides, *trim14* overexpression inhibited MESC ectodermal differentiation, resulting in decreasing number of the formed neurons (Novosadova et al., 2005).

To evaluate molecular mechanisms involving the *trim14*, we analyzed the transcriptome of ES-hPub (MESC transfected with *trim14*) and revealed a set of genes induced by *trim14* in embryonic cells. In addition, the research was accentuated on the genes proved to be connected with *pu.1* (Steidl et al., 2006), because, as it was mentioned above, TRIM14 inhibited the transcriptional activity of the Pu.1 (Hirose et al., 2003). Our present research revealed a number of genes upregulated at the *trim14* overexpression: *hsp90ab1*, *hbp1*, *prr13, pu.1, tnfrsf13c (baff-r), junb, tnfrsf13b (taci), hlx1, pdgfrb*.

Furthermore, we studied changes in gene expression in ES-hPub at early stage of cell differentiation (EB formation). Previously we found that EBs were formed one day earlier in cell line ES-hPub than in control ES-DNA3 cell line (Novosadova et al., 2012). Present research showed that the pattern of gene expression significantly changed at EB formation. Expression of *hbp1*, *ganab, prim2*, *tipin, c-jun, egr1, evi5, fosb, rhob, socs2* increased, while the expression level of *hsp90ab1*, *prr13, tnfrsf13c (baff-r), tnfrsf13b (taci), hlx1, pu.1, pdgfrb* decreased at EB formation. The only one gene, *junb*, showed the same enhanced expression before and after EB formation.

In order to understand whether the genes connected with *trim14* in MESC can participate in the processes mediated by *trim14* in other types of cells, we obtained human embryonic kidney cells (HEK)293T (the cells at later stage of differentiation) with overexpression of the *trim14* (HEK-

trim14). In these cells the expression levels were found to be enhanced as in ES-hPub for the genes *hsp90ab1*, *prr13*, *pu.1*, *tnfrsf13c (baff-r), tnfrsf13b (taci)* and *hlx.*

Thus, our investigation revealed upregulation of several common genes under *trim14* overexpression in embryonic cells of different origin and at various stages of differentiation. Taking into account the known data (as we described below) one may suppose an involvement of most of these genes in the process of mesodermal differentiation.

As it was shown above, the expression of *hbp1* is greater in undifferentiated cells ES-hPub and increases during EB formation. This observation may also be associated with the increased differentiation potential of MESC in the mesodermal direction under overexpression of *trim14*. Yao et al. (2005) reported that the enhanced *hbp1* expression directed the differentiation of myeloblast cell line K562 toward erythroid and megakaryocyte pathway. Moreover, the authors demonstrated that enhanced expression of *hbp1* was accompanied by an increase in *junB* mRNA. The latter also is upregulated in undifferentiated cells ES-hPub and EB in our study. In its turn, *junB* is a positive regulator of myeloid differentiation (Lord et al., 1993). Moreover, the JunB protein is a transcription factor and plays an essential role for the cells of the immune system (Su et al., 2012; Pekarsky et al., 2010).

In more differentiated HEK-*trim14* the absence of changes in the transcription of *hbp1* and *junB* supposes their involvement in the processes triggered by *trim14* only at the initial stages of cell differentiation. The same is true for *pdgfrb* upregulated in undifferentiated MESC and during EB formation but unchanged in more differentiated cells HEK-*trim14*. PDGFRB (β chain of the platelet-derived growth factor receptor) is a marker of pericytes, and, as it was shown recently, plays essential role in their maturation (Hamdan et al., 2011). Besides, *pdgfrb* is involved in human ESC differentiation in mesodermal direction (Hewitt et al., 2012).

For *prr13* (*txr1*), *hsp90ab1*, *hlx1*, *tnfrsf13c (baff-r)* and *tnfrsf13b (taci)* genes the other pattern of expression changes under *trim14* overexpression was observed: their expression levels increase in undifferentiated cells ES-hPub and in differentiated line HEK 293T, but not during EB formation.

As it was reported (Lih et al., 2006), *prr13* (*txr1*) negatively regulates expression of thrombospondin-1 (TSP-1) at transcription level. TSP-1 was known for both its anti-angiogenic and proapoptotic actions. Several publications state TSP-1 to be involved in the development and migration of pericytes (Canfield et al., 1996; Scheef et al., 2009). This fact allows us to suggest the similar involvement of *prr13* in these processes.

The *hsp90ab1* encodes the heat shock protein HSP90AB1 that belongs to chaperons. It is involved in different cellular processes, e.g. in angiogenesis and osteoclastogenesis (Okawa et al., 2009) and in erythoid differentiation (Morceau et al., 2008). Inhibition of HSP90AB1 in various cell

types leads to suppression of *pu.1* (Morceau et al., 2008), *pdgfrb* (Lang et al., 2009), that suppose an interrelation between these genes and *trim14*.

The genes *tnfrsf13c (baff-r)* and *tnfrsf13b (taci)* encode the proteins BAFFR and TACI, receptors for the B-cell-activating factor (BAFF) and for the proliferation-inducing ligand (APRIL), and play key roles in survival and proliferation of peripheral B-lymphocytes and plasma cells. The BAFF receptor is expressed practically at all stages of B-cell maturation. The receptor TACI is expressed only in mature B-cells, memory cells, germinal center cells and plasma cells, but not in immature lymphocytes. The BAFF binds with both receptors (BAFF-R and TACI), while APRIL binds only with TACI. By binding with the receptors, through activating the signal NF-κB pathways, BAFF can enhance expression of the antiapoptotic genes *bcl-2* and *bcl-XL* and suppress the proapoptotic gene *bim*, protecting B-lymphocytes from apoptosis (Su et al., 2012; Hayden and Ghosh, 2011). It was shown that TACI and BAFF-R are involved in differentiation of the adipose tissue-derived mesenchymal cells (ADMC) into adipocytes and probably into endothelial cells (Alexaki et al., 2009).

Hlx1 encodes a transcription factor that is actively involved in the process of placenta maturation (Rajaraman et al., 2008). It is also involved in myeloid maturing (Halene et al., 2010), and in differentiation of the T-lymphocytes (Thieu et al., 2008).

Summarizing the data concerning this group of genes (*prr13* (*txr1*), *hsp90ab1*, *hlx1*, *tnfrsf13c (baff-r)* and *tnfrsf13b (taci)*) we may assume their participation in new pathway connected with *trim14*-directed processes as in MESC as in HEK293T cells.

Among the genes that are upregulated at *trim14* overexpression only during EB formation, some may be involved in cell differentiation (*fosb* (Haasper et al., 2008), *egr1* (Gabet et al., 2010)), while others are connected with replication (*tipin* (Errico and Costanzo, 2012), *prim2* (Schneider et al., 1998) or transcription (*c-myc* (Lüscher and Vervoorts, 2012)). All these processes are known to be enhanced during EB formation.

In sum, we found a number of genes (*hsp90ab1*, *prr13*, *pu.1*, *tnfrsf13c (baff-r)*, *tnfrsf13b (taci), hlx1, hbp1, junb, pdgfrb*) being upregulated under *trim14* overexpression at MESC and/or HEK293T. All of them may be involved in cell differentiation in different extent. Our results suggest direct or indirect involvement of these genes in differentiation process of embryonic cells in the mesodermal direction mediated by *trim14*.

According to the well-known investigations (Abujarour et al., 2010) PU.1 induces differentiation of the ESC. Moreover, PU.1 regulates the development of myeloid and lymphoid cells (Gallant and Gilkeson, 2006). It was also established that the mouse homolog of the protein Trim14 (PUB) bounds with protein PU.1 and inhibits its activity (Hirose et al., 2003). Hirose et al. (2003) also observed that both *trim14* and *pu.1* were expressed in primary B-lymphocytes at various stages of

differentiation (Hirose et al., 2003). In the present work we show that the overexpression of *trim14* provokes the small but statistically significant increase in *pu.1* mRNA before differentiation and during the EB formation in MESC and in human HEK293T cells. This fact possibly indicates the cooperative activity of these genes in certain cases during a differentiation of embryonic cells. *Trim14* as we showed earlier enhances the MESC differentiation in lymphoid direction (Novosadova et al., 2009). We suppose that observed in the present work increase of the amount of *pu.1* mRNA at *trim14* overexpression may be connected with this process.

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