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GATA1 transcription factor targets the gene expression of B19 virus in HEK293 cell line

Amir Atashi[a], Leila Jafaripour[b], Kobra Froughi[c], Mahin Behzadifard[d,*]

Background/Aim: B19 virus (B19V) is a single-strand DNA virus that has specific tropism to erythroid progenitor cells (EPCs). The virus enters the cells via P antigen and coreceptors and induces infection and cell apoptosis. GATA1 has a high expression in EPC and is a critical transcription factor for the cells development and differentiation. As human EPCs are the main target of the virus infection that have high expression of GATA-1 as the critical transcription factor, the aim of this study was to investigate the effect of GATA1 cotransfection with B19V genome on the expression of the viral mRNAs in HEK293 as nonpermissive cell line to the virus that had no mRNA expression of GATA-1.

Methods: HEK293 cells were transfected with pHI0 plasmid containing the B19V genome and the plasmid of the GATA1 genome. The quantity of B19V mRNAs (NS1, 7.5 kDa, and 11 kDa) expression was evaluated after 24 h of transfection.

Results: The results showed a statistically significant increase in fold change expression of (NS1 ∽12.3, VP1 ∽27.6, 11kb protein ∽38) in cotransfected cells with GATA1 and B19 plasmids compare to control group ($P < 0.05$).

Conclusion: This research showed transfected cells with GATA1 had elevation in the expression of the B19V genes mRNAs in a nonpermissive cell. This result may show the role of GATA1 as a critical transcription factor in support of the virus infection in EPCs. This suggests that GATA1 may potentially sport B19V replication or gene expression.

Keywords: B19V, EPCs, erythroid progenitor cells lineage, GATA1, HEK293, NS1, parvovirus B19, permissive cell, transfection

Introduction

B19 virus (B19V) is a nonenveloped, single-stranded DNA virus from the genus of the Erythrovirus and Parvoviridae family[1]. Nonstructural proteins that have roles in the virus pathogenesis, including NS1, 11kDa, and 7.5kDa, and structural proteins that produce the viral capsid, encompass VP1 and VP2 encoded by this virus genome. NS1 regulates DNA replication, transcription, induces IL-6 and proinflammatory cytokine production, and damage and apoptosis in the infected cells[2,3]. By age 15 ~50% of children are seropositive for the virus. Erythema infectiosum, arthropathies, myocarditis, transient aplastic crisis, fetal death and/or hydrops fetalis[4,5]. P antigen and both Ku70/80 and α5b1 integrin are the main and coreceptors of the virus[6]. Erythroid progenitor cells (EPCs) are the main target that are permissive to the virus and support the infection[7,8]. Permissive cells expressed the B19V receptor and coreceptors are permeable to entrance of the virus but may be unable to support the virus replication and infection[9–11]. In nonpermissive cells, the B19V genome may be detected, and viral particle endocytosis is suggested for the virus entrance in these cells, but the virus can induce no infection in the infected cells. Previously we and others showed that this inhibi tion might be due to specific microRNAs (miRNAs) that pre sented in nonpermissive cells gene expression[9,12]. GATA1 is one of the main transcription factors in the regulation of erythroid cells gene expression[13,14]. The death of erythroid progenitors occurred in embryos with the failure in GATA1 transcription factor expression[15]. B19V NS1 modulates GATA1 and GATA2 expression that may be a potential mechanism in inducing B19V-related anemia. NS1 inhibits the differentiation of hema topoietic stem cells into mature normocytes by downregulating GATA1 and upregulating GATA2 transcription. GATA1 con tributes to erythroid maturation, but GATA2 is predominantly

HIGHLIGHTS

- B19 virus (B19V) is a single-strand DNA virus that has specific tropism to erythroid progenitor cells (EPCs).
- Permissive cells expressed by the B19V receptor and coreceptors are permeable to the entrance of the virus but may be unable to support the virus replication and infection.
- GATA1 has a high expression in EPCs and is one of the main transcription factors in the regulation of erythroid cells gene expression. Cotransfection of GATA1 and B19 genome in HEK293 as a nonpermissive cell to the virus leads to the expression of the viral proteins mRNAs.

[a]Department of Tissue Engineering, School of Medicine, Shahroud University of Medical Sciences, Shahroud, Iran, [b]Department of Anatomy, School of Medical Sciences, Dezful University of Medical Sciences, Dezful, Iran, [c]Department of Biotechnology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran and [d]Department of Laboratory Hematology and Blood Banking, Dezful University of Medical Sciences, Iran

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*Corresponding author. Address: Department of Laboratory Sciences, School of Allied Medical Sciences, Dezful University of Medical Sciences, Dezful, Iran. E-mail: mahinbehzadi2020@gmail.com (M. Behzadifard).

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expressed in hematopoietic stem cells and EPCs and plays an important role in these cells' development and maintenance. Disrupting the balance of the 'GATA switch' can stop erythroid differentiation. In K562 cells, NS1 triggers the Notch signaling pathway and disrupted the 'GATA switch' balance. Cytoplasmic NS1 activates Notch1 signaling to inhibit GATA1 expression and nuclear NS1 directly upregulates Hes1 expression to further suppress GATA1. These findings clarify the molecular underlying mechanisms in the virus pathogenesis and can help to introduce therapeutic strategies for the virus-related hematological disorders[16].

Erythropoietin signaling is an important pathway in EPCs maturation to erythrocytes and is an as crucial factor in enhancing B19V replication. In this pathway activation of signal transducer and activator of transcription 5 (STAT5) plays a direct role in B19V DNA replication. Pimozide, an FDA-approved drug that dephosphorylates STAT5, inhibits B19V replication in EPCs and can be used for the virus-related diseases[17].

B19V infection in fetus induce failure in hematopoiesis by inducing maturation and differentiation arrest in erythroid progenitor cells[16]. As previous reports showed the role of GATA1 in the erythroid development and differentiation and may be support to induce B19V infection, we speculate that GATA1 may lead to B19V genes expression. In this study, we used the GATA1 vector in nonpermissive cells infected with the B19V vector and analyzed the virus gene expression.

Materials and methods

Screening analysis of transcription factor binding sites (TFBS)

NS1 is synthesized from a strong promoter in the left side of map unit 6 (p6) of the virus genome. It represents a significant role in B19V replication, transcription, and the activation of the B19V via p6 promoter[18,19]. To retrieve the promoter sequences for further analysis, a 615 bp 5'-flanking sequence of B19V was used from GenBank accession number AF162273 as a complete genome (https://www.ncbi.nlm.nih.gov/nuccore/AF162273.1). The promoter sequences were analyzed to predict potential transcription factor binding sites by using the program TFSEARCH (http://diyhpl.us/~bryan/irc/protocol-online/protocol-cache/TFSEARCH.html), TFBIND (https://tfbind.hgc.jp/). The promoter region of human parvovirus B19V was determined by exploring for putative transcription factors and binding sites using a transcription factor binding site profile database TFSEARCH and TFBIND (Fig. 1). The analysis of promoter regions showed that two regions of the promoter, from −568 to −559 and −347 to −338 region of the P6 promoter contain binding sites for GATA1.

Cell culture

HEK293 cells (human embryonic kidney cells) were obtained from the Cell Bank (Pasture Institute) and cultured in RPMI-1640 medium (Gibco) containing 10% fetal bovine serum (Gibco) and 100 mg/ml streptomycin, 10 U/ml penicillin and incubated under standard conditions (37°C, 5% CO_2) for 1 week. Every 3 days culture replacement was performed.

Transfection

Transfection of pHI0 plasmid and plasmid containing GATA1 gene (1.5 μg) was performed in 5×10^5 HEK293T cells. After running the program, the cells were transferred into 1000 μl RPMI-10% FBS. The 6-well content was divided into 300 μl volumes in a 12-well plate, respectively, and incubated in a humidified 37°C/5% CO_2 incubator. For mock (without any of the vectors) and controls (transfected only by PHI0 or GATA1), the same condition of transfection were used. GFP groups transfected by pmaxGFP were used to assess the transfection efficiency. The cells were harvested 24 h after transfection. The tests were performed in triplicate.

Investigation of HEK293 cells transfection efficiency

The efficiency of transfection was assessed after 24 h by fluorescence microscopy and flowcytometric analysis on GFP-transfected HEK293T cells (24 h post transfection) and HEK293 cells without pmax GFP transfection. For flowcytometry analysis, 50×10^3 cells from each group were transferred into 2 ml microtubes with 100 μl phosphate-buffered saline (PBS, Sigma) with 0.5% BSA.

The genes expression assay

To quantify the genes expression qRT-PCR was performed. Total RNA was isolated from cultured cells by using TRIzol reagent (Biofact), and cDNA was synthesized from 1 μg of total RNA (Amplicon). SYBR Green Master (Rox) was used for qRT-PCR (BIORAD), 2.0 μl of cDNA, 10.0 μl of 2X SYBR PCRMix, 7.2 μl nuclease-free water and primers at a final concentration of 0.2 μM in a volume of 20.0 μl for each sample of qRT- PCR. GATA1 used primers was; forward: 5′ATGCCTGTAATCCCAGCACT3′ and reverse: 5′TCATGGTGGTAGCTGGTAGC-3′. GAPDH (ΔCt) was used to normalize relative target genes expression; fold changes in the target genes mRNA expression were calculated using $2^{-\Delta\Delta ct}$. All samples and control were assayed in triplicate. The primers sequences used to amplify the target genes in this study are shown in Table 1[18].

Results

GATA1 gene expression

RT-PCR was performed for the cells transfected with GATA1 and PHI0 and control groups that were transfected only by PHI0 or GATA1 and mock cells, without any of the vectors transfection. The results showed expression of GATA1 in the group cotransfected with GATA1 and PHI0, and GATA1 only transfected group and no expression in mock and PHI0 groups.

The efficiency of HEK293 cells transfection

The transfection efficiency of the transfected cells by GFP vector by flowcytometry showed, efficiency > 90% compared to 3.8% for the cells without the vector (Fig. 2).

Expression of the target genes

Our results showed a significant increase in fold change expression of (NS1 ∽12.3, VP1 ∽27.6, VP1-2 ∽3, 11kb protein ∽38) in GATA1 transfected cells compared to the control group ($P < 0.05$).

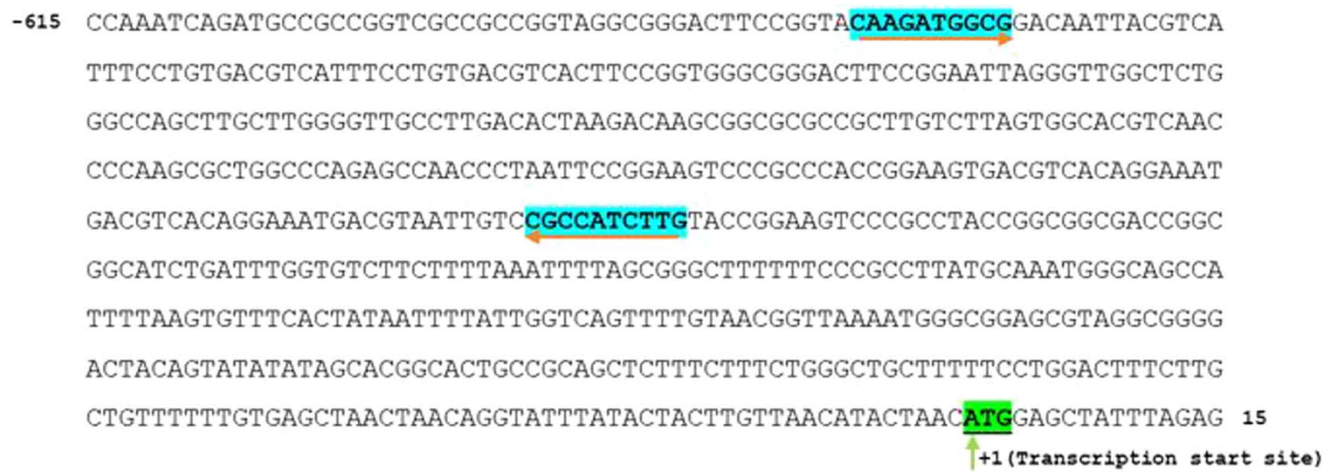

Figure 1. GATA1 binding sites within the P6 promoter region -615 bp to the transcription start site was identified using TFSEARCH and TFBIND databases.

Discussion

NS1, 11kDa, and 7.5kDa proteins are nonstructural, and VP1 and VP2 are capsid proteins encoded by the B19V genome. NS1 regulates DNA replication and transcription, induces IL-6 and proinflammatory cytokines production and induce damage and apoptosis in the infected cells[2,3].

NS1 and 11-kDa protein of B19V aids the virus replication. Their study revealed a novel role for the 11-kDa protein in regulating viral DNA replication. They found that 11-kDa interacts with cellular growth factor receptor-bound protein 2 (Grb2) and disruptes extracellular signal-regulated kinase (ERK) signaling and increases the virus replication[20].

B19V activates a DNA damage response (DDR) through critical kinases such as ATR, ATM, and DNA-PKcs in the infected cells that are facilitated by viral DNA replication mainly through NS1. This DDR seems to help B19 replication, subsequently, NS1intracts with E2F and induces G2 phase cell cycle arrest, viral genome itself induce S-phase arrest in EPCs and erythropoiesis. B19V activates cell death and apoptosis in EPCs via intrinsic and extrinsic pathways through caspase and DNA fragmentation pathways. In infected cells besides NS1, B19- 11kDa protein is involved in the induction of cell death[21].

Table 1

For each primer pair, sense (+) and antisense (–) primers are indicated.

Primer	Position	Sequence (5′–3′)	Target (genome region)
HR1 +	1882–1901	GCGGGAACACTACAACAACT	NS protein
HR1 –	2033–2014	GTCCCAGCTTTGTGCATTAC	NS protein
HR2 +	2210–2229	CGCCTGGAACACTGAAACCC	Common exon
HR2 –	2355–2336	GAAACTGGTCTGCCAAAGGT	Common exon
HR3 +	2915–2934	GCAGTCATGCAGAACCTAGA	VP1 protein
HR3 –	3044–3025	GGCCCAGCTTGTAGCTCATT	VP1 protein
HR4 +	4812–4831	GAATCCTCAACCTGGAGTAT	VP1-2 proteins
HR5 +	4899–4918	ACACCACAGGCATGGATACG	VP1-2, 11 kDa proteins
HR4/5 –	5022–5003	GGTACTGGTGGGCGTTTAGT	VP1-2, 11 kDa proteins
HR6/7 +	547–566	CCTGGACTTTCTTGCTGTTT	Leader sequence
HR6 –	2317–2298	CACGATGCAGCTACAACTTC	Central exon
HR7 –	4980–4961	GGTGGGGAGTGTTTACAATG	Distal exon

Position and sequence are referred to as sequence NC_000883 in the NCBI Genome Database.

B19V has a specific tropism to EPCs and can induce erythroid maturation arrest in cases with failure in erythropoiesis[4,5].

The first expression of the GATA1 transcription factor occurs in common myeloid progenitor and then increases in burst-forming unit-erythroid (BFU-E) and colony-forming unit-erythroid (CFU-E) phases gradually and reaches a peak at the pronormoblast stage, and then decreases in more matured erythroid precursors[22–26]. Previous studies have been shown that GATA1 is an important transcription factor in supporting B19V infection in erythroid cells lineage, and the inhibitory effect of NS1 on GATA-1 can lead to decrease in the proliferation and differentiation of the erythroid lineage[16,27,28].

The study by Bartunek *et al.* (2003) explored the relationship between GATA-1 and c-Myb transcription factors in red cell development using an in vitro culture system. They found that as cells differentiated, GATA-1 expression increased, and leading to the accumulation of nuclear GATA-1 protein. This increase in GATA-1 expression coincided with a decrease in c-myb expression levels. Through their experiments, they identified two binding sites for GATA-1 within the c-myb promoter and demonstrated that GATA-1 directly repressed c-myb expression at a specific binding site, a process requiring the FOG-1 protein. These findings provide direct molecular evidence of the regulatory link between GATA-1 activity and c-myb proto-oncogene expression during the terminal differentiation of red cells[29].

Chen and colleagues delved into the relationship between FAM122A and GATA-1. While FAM122A is widely conserved, its exact functions remain elusive. It exhibits heightened expression in early erythroid cells but diminishes as erythroid differentiation progresses. Their research revealed that increasing FAM122A levels inhibits the generation of red blood cells in both primary human hematopoietic progenitor cells and erythroleukemia cells. They also found that FAM122A directly interacts with the C-terminal zinc finger domain of GATA1, a critical regulator of erythropoiesis, leading to a reduction in GATA1's binding to its target gene promoters and a subsequent decrease in its transcriptional activity. These findings underscore the inhibitory role of FAM122A in regulating erythroid differentiation and suggest

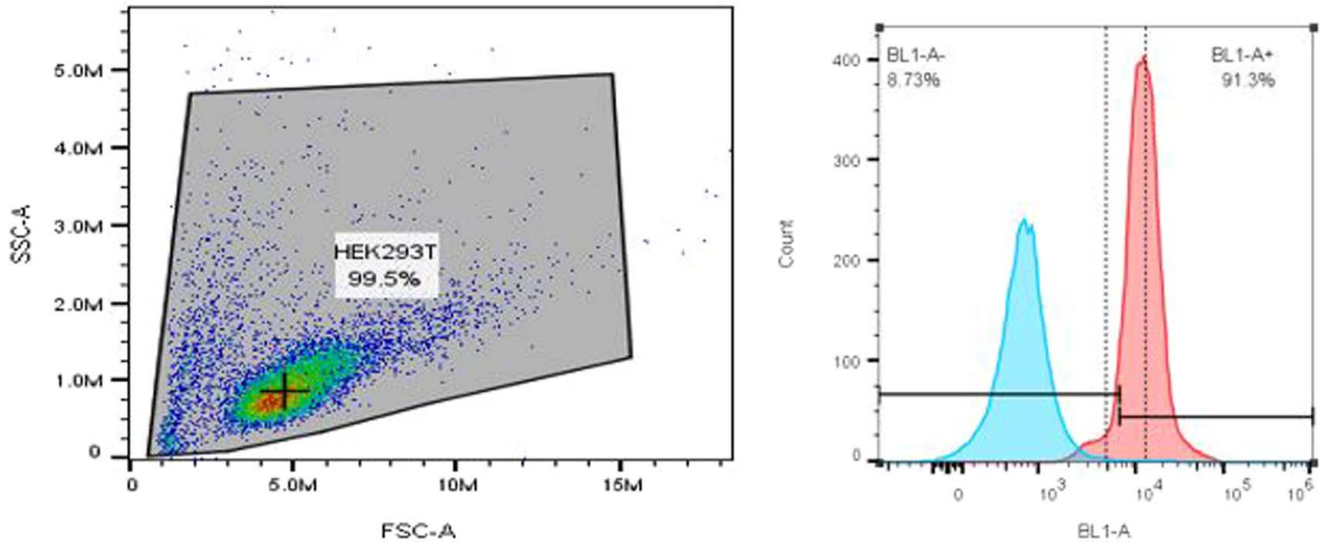

Figure 2. The analysis of the results of fluorescence flowcytometry results showed >90% of the transfected cells were fluorescent-positive.

its potential as a therapeutic target for conditions associated with GATA1 dysfunction, or as a crucial regulator for expanding red blood cells in laboratory settings[30].

We previously showed B19V decreases the BFU-E and CFU-E progenitors that produced by hematopoietic stem cells that were cocultured with infected mesenchymal stem cells with the virus[3]. As reported in previous studies, HEK293 express no GATA1 and are nonpermissive to the virus[31]. Our study showed elevation of the B19V-related mRNA proteins mRNAs in trans fected HEK293 cells with GATA1 vector. The findings can emphasis the role of this transcription factor in support of B19V infection in permissive cells to the virus. It paves the way for fur ther research to elucidate the precise mechanisms by which GATA1 interacts with the B19V genome and its potential role in the pathogenesis of B19V-related erythroid and hematological diseases.

Ethical approval

This study was approved by the Research Ethics Committee of Dezful University of Medical Sciences (ethical approval code: IR.DUMS.REC.1402.016).

Consent

Not applicable.

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Author contribution

A.A. and K.F.: designed the project and did experiments; M.B. and L.J.: wrote the manuscript. All authors read the final manuscript and approved it.

Conflicts of interest disclosure

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Research registration unique identifying number (UIN)

Not applicable.

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Data availability statement

Datasets generated during and/or analyzed during the current study are publicly available.

Provenance and peer review

The authors declare that they have no conflict of interest to the publication of this article .The manuscript has been seen and approved by all authors and is not under active consideration for publication. It has neither been accepted for publication nor published in another journal fully or partially. Corresponding

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References

[1] Cotmore SF, Agbandje-McKenna M, Chiorini JA, *et al*. The family parvoviridae. Arch Virol 2014;159:1239–47.
[2] Fard MB, Atashi A, Amiri S, *et al*. Parvovirus B19 affects thrombopoietin and IL-11 gene expression in human bone marrow mesenchymal stem cells. Future Virol 2021;16:519–26.
[3] Behzadi Fard M, Kaviani S, Atashi A. Parvovirus B19 infection in human bone marrow mesenchymal stem cells affects gene expression of IL-6 and TNF-α and also affects hematopoietic stem cells differentiation. Indian J Hematol Blood Transfus 2019;35:765–72.
[4] Gallinella G. Parvovirus B19 achievements and challenges. Int Schol Res Notic 2013;2013. https://api.semanticscholar.org/CorpusID:84592174
[5] Prasad N, Novak JE, Patel MR. Kidney diseases associated with parvovirus B19, Hanta, Ebola, and Dengue virus infection: a brief review. Adv Chron Kidney Dis 2019;26:207–19.
[6] Munakata Y, Saito-Ito T, Kumura-Ishii K, *et al*. Ku80 autoantigen as a cellular coreceptor for human parvovirus B19 infection. Blood 2005;106: 3449–56.
[7] Weigel-Kelley KA, Yoder MC, Srivastava A. α5β1 integrin as a cellular coreceptor for human parvovirus B19: requirement of functional activation of β1 integrin for viral entry. Blood 2003;102:3927–33.
[8] Brown KE, Anderson SM, Young NS. Erythrocyte P antigen: cellular receptor for B19 parvovirus. Science 1993;262:114–7.
[9] Berillo O, Khailenko V, Ivashchenko A, *et al*. miRNA and tropism of human parvovirus B19. Computat Biol Chem 2012;40:1–6.
[10] Guan W, Cheng F, Yoto Y, *et al*. Block to the production of full-length B19 virus transcripts by internal polyadenylation is overcome by replication of the viral genome. J Virol 2008;82:9951–63.
[11] Winter K, von Kietzell K, Heilbronn R, *et al*. Roles of E4orf6 and VA I RNA in adenovirus-mediated stimulation of human parvovirus B19 DNA replication and structural gene expression. J Virol 2012;86:5099–109.
[12] Anbarlou A, AkhavanRahnama M, Atashi A, *et al*. Possible involvement of miRNAs in tropism of Parvovirus B19. Mol Biol Rep 2016;43:175–81.
[13] Crispino JD, Horwitz MS. GATA factor mutations in hematologic disease. Blood 2017;129:2103–10.
[14] Richards LA, Kumari A, Knezevic K, *et al*. DKC1 is a transcriptional target of GATA1 and drives upregulation of telomerase activity in normal human erythroblasts. Haematologica 2020;105:1517.
[15] Fujiwara Y, Browne CP, Cunniff K, *et al*. Arrested development of embryonic red cell precursors in mouse embryos lacking transcription factor GATA-1. Proc Natnl Acad Sci 1996;93:12355–8.
[16] Zeng D, Zheng J, Feng S, *et al*. Human Parvovirus B19 Nonstructural Protein 1 Regulates GATA1 Expression via the Notch Signaling Pathway in K562 Cell Line. Front Biosci-Landmark 2022;27(9)):261.
[17] Ganaie SS, Zou W, Xu P, *et al*. Phosphorylated STAT5 directly facilitates parvovirus B19 DNA replication in human erythroid progenitors through interaction with the MCM complex. PLoS Pathogens 2017;13:e1006370.
[18] Brown KE, Green SW, de Mayolo JA, *et al*. Congenital anaemia after transplacental B19 parvovirus infection. The Lancet 1994;343: 895–6.
[19] Doerig C, Hirt B, Antonietti J-P, *et al*. Nonstructural protein of parvoviruses B19 and minute virus of mice controls transcription. J Virol 1990; 64:387–96.
[20] Xu P, Chen AY, Ganaie SS, *et al*. The 11-kilodalton nonstructural protein of human parvovirus B19 facilitates viral DNA replication by interacting with Grb2 through its proline-rich motifs. J Virol 2019;93:e0146418.
[21] Qiu J, Söderlund-Venermo M, Young NS. Human parvoviruses. Clin Microbiol Rev 2017;30:43–113.
[22] Bonvicini F, Filippone C, Delbarba S, *et al*. Parvovirus B19 genome as a single, two-state replicative and transcriptional unit. Virology 2006;347: 447–54.
[23] Moriguchi T, Yamamoto M. A regulatory network governing Gata1 and Gata2 gene transcription orchestrates erythroid lineage differentiation. Int J Hematol 2014;100:417–24.
[24] Rogo L, Mokhtari-Azad T, Kabir M, *et al*. Human parvovirus B19: a review. Acta Virol 2014;58:199–213.
[25] Lamont RF, Sobel J, Vaisbuch E, *et al*. Parvovirus B19 infection in human pregnancy. BJOG 2011;118:175–86.
[26] Feng S, Zeng D, Zheng J, *et al*. New insights of human parvovirus B19 in modulating erythroid progenitor cell differentiation. Viral Immunol 2020;33:539–49.
[27] Chen AY, Zhang EY, Guan W, *et al*. The small 11kDa nonstructural protein of human parvovirus B19 plays a key role in inducing apoptosis during B19 virus infection of primary erythroid progenitor cells. Blood 2010;115:1070–80.
[28] Fryer CJ, Lamar E, Turbachova I, *et al*. Mastermind mediates chromatin-specific transcription and turnover of the Notch enhancer complex. Genes Developm 2002;16:1397–411.
[29] Bartůněk P, Králová J, Blendinger G, *et al*. GATA-1 and c-myb crosstalk during red blood cell differentiation through GATA-1 binding sites in the c-myb promoter. Oncogene 2003;22:1927–35.
[30] Chen J, Zhou Q, Liu MH, *et al*. FAM122A inhibits erythroid differentiation through GATA1. Stem Cell Rep 2020;15:721–34.
[31] Mózner O, Zámbó B, Sarkadi B. Modulation of the human erythroid plasma membrane calcium pump (PMCA4b) expression by polymorphic genetic variants. Membranes 2021;11:586.