FOLR1-induced folate deficiency reduces viral replication via modulating APOBEC3 family expression

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Abstract

Folate receptor alpha (FOLR1) is vital for cells ingesting folate (FA). FA plays an indispensable role in cell proliferation and survival. However, it is not clear whether the axis of FOLR1/FA has a similar function in viral replication. In this study, we used vesicular stomatitis virus (VSV) to investigate the relationship between FOLR1-mediated FA deficiency and viral replication, as well as the underlying mechanisms. We discovered that FOLR1 upregulation led to the deficiency of FA in HeLa cells and mice. Meanwhile, VSV replication was notably suppressed by FOLR1 overexpression, and this antiviral activity was related to FA deficiency. Mechanistically, FA deficiency mainly upregulated apolipoprotein B mRNA editing enzyme catalytic subunit 3B (APOBEC3B) expression, which suppressed VSV replication in vitro and in vivo. In addition, methotrexate (MTX), an FA metabolism inhibitor, effectively inhibited VSV replication by enhancing the expression of APOBEC3B in vitro and in vivo. Overall, our present study provided a new perspective for the role of FA metabolism in viral infections and highlights the potential of MTX as a broad-spectrum antiviral agent against RNA viruses.

Introduction

Folate receptors (FOLRs) are high-affinity transporters for folate (FA) and include four members named FOLR1–4 (Karampelias et al., 2021; Nawaz and Kipreos, 2022). The expression of FOLR1 is generally restricted to the luminal surface including lungs, proximal kidney tubules, ovary, etc (Cao et al., 2021; Karampelias et al., 2021; Bax et al., 2023). FOLR1 bound to FA and acted as the transfer of FA across cellular barriers via endocytosis pathway, which was important for cellular uptake and utilization of FA (Nawaz and Kipreos, 2022).

Benzo(a)pyrene (Bap) is one of the key components in cigarette smoke (CS) and widely present in human daily life (Shi et al., 2017). Bap enters into the human body through skin, respiratory and digestive tracts, causing a broad array of health problems (Herrmann et al., 2020). FA deficiency perturbs virus maturation after HPV initial infection (Pathak et al., 2018). Meanwhile, the relationship between FA metabolism and viral infection is complex and the underlying mechanisms remain unclear. Thus, it is necessary to investigate the mechanisms between FA metabolism and viral replication to explore new antiviral targets.

Keywords:
Folate receptor alpha (FOLR1)
Folate receptor
Vesicular stomatitis virus (VSV)
Apolipoprotein B mRNA editing enzyme catalytic subunit 3 (APOBEC3)
Methotrexate (MTX)

1. Introduction

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APOBEC3B enzymes often induce genomic mutations that are involved in antiviral immunity and tumorigenesis (Faure-Dupuy et al., 2021). Previous studies showed that the APOBEC3 protein family is important in the restriction of retroviruses (Harris and Liddament, 2004), herpesviruses (Huff et al., 2018), hepatitis viruses (Supsene et al., 2011), coronavirus (Milewska et al., 2018) and cellular RNA substrates synthesis (Sharma et al., 2015; Milewska et al., 2018). In addition, APOBEC3B can efficiently recognize and deaminate deoxyctydine (dC) whose neighboring nucleotides are damaged and continue to mediate nucleotide damage (Diamond et al., 2019). However, it is not clear at the present how FA regulates the expression of APOBEC3B.

Vesicular stomatitis virus (VSV), a member of Rhabdoviridae family, is transmitted through aerosol and infects the upper respiratory tract (Lichty et al., 2004). Meanwhile, VSV is well characterized to study immune responses (Wu et al., 2020). Therefore, we used VSV in our current study to investigate the relationship between FOLR1-mediated FA deficiency and viral replication, to explore the underlying mechanisms, and to find new targets and potential interventions in viral infectious diseases.

2. Materials and methods

2.1. Preparation of solution

Bap standard (3,4-Benzopyrene) was obtained from MACKLIN (Cas. No. 50-32-8; MACKLIN, China). FA (Cas. No. 59-30-3) and MTX (Cas. No. 59-05-2) were also purchased from MACKLIN (Shanghai, China). The powders of Bap, FA, and MTX were weighed and dissolved in DMSO (Cas. No. 67-68-5; Solarbio, China), and the solutions were prepared for stock and stored away from light. In vivo, the stock solution of Bap was prepared in 0.5% DMSO, 5% Tween 20 and 94.5% PBS for intraperitoneal administration in mice (Yi et al., 2019). The stock solution of MTX was prepared in corn oil for intraperitoneal injection in mice.

2.2. Cells, viruses and animals

Human cervical cancer cells (HeLa), human pulmonary epithelial cell (A549), and African green monkey kidney cells (Vero E6) were purchased from American Type Culture Collection (ATCC) and were grown in DMEM (Gibco) with 10% heat-inactivated FBS (Gibco). Human laryngeal cancer epithelial cells (Hep2) cells were cultured in RPMI 1640 (Gibco) with 10% heat-inactivated FBS. All cells were cultured at 37 ℃ in an incubator containing 5% carbon dioxide.

Specific pathogen-free (SPF) C57BL/6J mice (female, 6–week-old) were purchased from the Yangzhou University Center for Comparative Medicine (Yangzhou, China). All animal experiments were approved by the Nanjing University Animal Care Committee and were conducted in strict accordance with the Guide for the Care and Use of Laboratory Animals published by the Chinese National Institutes of Health. The research protocols followed relevant policies regarding animal handling as mandated under the guidelines from the institutional animal care committee.

Vesicular stomatitis virus (VSV) Indiana strain was purchased from Imanis Life Sciences (USA); respiratory syncytial virus (RSV) was a gift from Professor Hongwei Wang, Nanjing University; enterovirus 71 (EV71) BrCr strain, coxsackie virus A16 (CA16), and fever with thrombocytopenia syndrome virus (SFTSV) HB29 strain were gifts from Professor Ping Wu, at the Jiangsu Provincial Center of Disease Control. VSV, EV71, CA16 and SFTSV were propagated in Vero E6 cells and RSV was propagated in Hep2 cells. The multiplicity of infection (MOI) was specified in each figure legend.

2.3. Treatment and siRNAs transfection

HeLa and A549 cells were pre-exposed to Bap at a concentration of 10 μmol/L for 48 h, followed by infection with VSV (MOI = 1) or RSV (MOI = 1) for 24 h in the presence of Bap. The working solution of FA at a concentration of 1 μmol/L was used for the entire Bap exposure process. HeLa cells were pretreated with 1 μmol/L MTX for 2 h, and then infected with VSV (MOI = 1), RSV (MOI = 1), EV71 (MOI = 1), CA16 (MOI = 1), and SFTSV (TCID50) for 24 h. The culture medium was replaced with fresh 2% FBS mediumdrug mixture daily. HeLa cells were treated with FA-free RPMI 1640 medium (Gibco) containing 2% heat-inactivated FBS for 72 h to establish an in vitro FA deficiency model.

APOBEC3B siRNA (siAPOBEC3B) and negative control siRNA (siNC) were synthesized by Genepharma (Shanghai, China). Lipo3000 reagent was used for siRNA transfection. 1.5 μL Lipofectamine reagent and 50 nmol/L siRNA were co-incubated in 125 μL Opti-MEM for 15 min at room temperature. Then they were co-cultured with cells in 1 mL basal medium for 36 h. The siRNA sequences are shown in Supplementary Table S1. All in vitro experiments were performed in three parallel experiments.

The C57BL/6J mice (female, 6-week-old) were randomly divided into 5 groups (n = 25). The mice were administered intranasally with or without Bap (0.5 μg/mouse) four times (on day 1, 4, 7, 10). The group of FA-supplemented mice was given 15 mg/L of FA in water throughout the experiment. The group of MTX-treated mice were pretreated with MTX (0.5 μg/mouse) for 72 h by intraperitoneal injection. And then the mice were infected with VSV (MOI = 1) for 36 h by intranasal delivery. The lungs and serum of the mice were collected, respectively.

2.4. Cell viability assay

Cell counting kit-8 (CCK-8) assays (Cat. No. C0037; Beyotime, China) were performed to detect cell viability. Cells were cultured in 96-well plates (5000 cells per well). After cells were treated, CCK-8 solution (100 μL) was added to each well. The absorbance was detected by a microplate reader (TECAN infinite M200) after 2 h of incubation. The cell viability was calculated as (ODtreatment − ODtreatment-blank)/(OD control − ODcontrol-blank) × 100%.

2.5. Real-time fluorescence quantitative polymerase chain reaction (qPCR)

After treatment, cells were washed three times with 1 × PBS. RNA was extracted by TRIzol reagent (Invitrogen, Shanghai, China) according to the manufacturer's instructions. RNA extract (2 μL) was reverse transcribed to cDNA in a total reaction volume of 20 μL using HiScript® III RT SuperMix for qPCR (Cat. No. R122-01; Aazyme Biotech, China). RT-qPCR were performed by ABI 7500 (USA), with 20 μL reaction mixtures containing 2 μL cDNA, 10 μL 2 × AceQ Universal SYBR qPCR Master Mix (Cat. No. Q511-02; Aazyme Biotech, China), 0.8 μL primers, and 7.2 μL sterilized water. The primer sequences used in this study were shown in Supplementary Table S2.

2.6. Western blot

Cells were lysed with RIPA lysis buffer (Cat. No. sc-24948A; Santa Cruz, USA) and total protein concentrations were determined by the BCA protein assay (Cat. No. 23225; Pierce Rockford, IL, USA). Equal concentrations of harvested protein were electrophoresed on 10% (w/v) sodium dodecyl sulfate (SDS) polyacrylamide gel and transferred onto a 0.45 μm PVDF membrane (Cat. No. 03010040001; Millipore, Billerica MA, USA) which was activated in methanol. The primary antibodies used to detect the target proteins were as following: anti-VSV-G was purchased from Abcam (Cat. No. ab183497; Cambridge, UK). Anti-RSV-G and anti-GFP were both purchased from Sino Biological (Cat. No. 13029-T25; Cat. No. 13105-R208; Beijing, China). Anti-FOLR1 and anti-APOBEC3B were purchased from Abclonal (Cat. No. A15672; Cat. No. A9010; Wuhan, China). Anti-GAPDH was purchased from Beyotime (Cat. No. AF0006; Shanghai, China). IRDye 680 goat-anti-rabbit and IRDye 800 goat-anti-mouse were purchased from Li-COR (Cat. No. 926-68071; Cat. No. 926-32216; Lincoln, NE, USA), and co-incubated with the membrane. Then they were visualized by a Li-COR Odyssey Infrared Imager. The
protein bands were quantified using ImageJ (National Institutes of Health and the Laboratory for Optical and Computational Instrumentation, university of Wisconsin, America).

2.7. Enzyme-linked immunosorbent (ELISA) assay

The levels of extracellular and intracellular human-FAnA, and mouse-FAnA in mouse serum were detected by ELISA kits purchased from X-Y Biotech (Cat. No. XYM903291; Cat. No. HZ-FA-HU; Shanghai, China). The experimental procedure was carried out strictly according to the manufacturer's instructions.

2.8. RNA sequencing (RNA-seq)

Bap-treated HeLa cells and control HeLa cells were subjected to mRNA sequencing by Lianchuan Biotech (Hangzhou, China). Illumina HiSeq sequencing platform was used. The cDNA library was constructed and sequenced in 2 × 150 sequencing mode. The quality of pre-processed data was assessed by FastQC software, and sequence match was performed with reference genomic sequences of the sequenced species using STAR software. HTSeq software and the FPKM indicator were used to calculate raw sequences and known gene expression. DESeq software was used to screen for differentially expressed genes (DEGs). The RNA-seq data were deposited in the National Center for Biotechnology Information Gene Expression Omnibus database with accession number GSE191225.

2.9. Fluorescence imaging

Green fluorescence of VSV-GFP was observed under a fluorescence microscope in vitro (200×) and in paraffin sections of lung tissue. Mice were sacrificed after isoflurane inhalation anesthesia, and lung tissue was immediately imaged for VSV-GFP using an animal imaging system (LB 983 NC100). Stronger fluorescence indicated a greater amount of VSV replication.

2.10. Statistical analysis

All data, except for RNA-Seq data, were analyzed using Prism 8.0 software (GraphPad Prism). Data were evaluated by nonparametric Wilcoxon rank test or one-way analysis of variance (ANOVA). Variables at different time points were assessed using repeated measures ANOVA. And then Bonferroni's correction was used for comparison of multiple means analysis. Error bars on all graphs and tables represent the standard error of the mean. Significance was defined as *P < 0.05, ***P < 0.01, or ****P < 0.001.

3. Results

3.1. Bap upregulated FOLR1 expression in vitro and in vivo

First, we would like to investigate the impact of the pollutant exposure on viral replication, and we observed that Bap suppressed the replication of VSV in vitro and in vivo (Supplementary Fig. S1). To explore the mechanism of Bap-mediated antiviral effects, we performed RNA sequencing analysis on Bap-stimulated HeLa cells. 866 genes were upregulated and 15 genes among them were significantly upregulated (|log2FC| ≥ 4) in Bap-stimulated HeLa cells as compared to the Mock control group (Fig. 1A and Supplementary Fig. S2A). Validation of these findings by qPCR analysis revealed that Bap exposure upregulated FOLR1, CN3N3, CR1, NQO1 and THBS1 mRNA expression in HeLa cells (Fig. 1B). Previous studies have shown a potential link between Bap exposure and FA supplementation (Zhang et al., 2016, Wang et al., 2019). Thus, we selected FOLR1, a receptor that mediates FA entry into cells, for further analysis. Our data confirmed that FOLR1 protein was upregulated by Bap stimulation (Fig. 1C). Furthermore, we observed an increase in FOLR1 mRNA and protein expression in lung tissues of mice exposed to Bap (Fig. 1D, E, and 1F). All these observations demonstrated that Bap induced upregulation of FOLR1 both in vitro and in vivo. However, the roles of FOLR1 playing in Bap-mediated suppression of VSV remains to be determined.

3.2. FOLR1-mediated FA deficiency was involved in reducing VSV replication

FOLR1 is a cysteine-rich cell-surface glycoprotein that binds FA with high affinity to mediate the cellular uptake of FA (Chen et al., 2013). FA uptake is vital for nucleic acid synthesis (Sobczyńska-Malefora and Harrington, 2018), which is a critical step in viral replication (Meng et al., 2019). Based on our findings, we hypothesize that Bap-induced upregulation of FOLR1 leads to FA deficiency, which leads to the activation of cellular mechanisms that impact on viral replication. Our data showed that the intracellular and extracellular FA were decreased significantly upon Bap stimulation for 72 h (Fig. 2A). Additionally, the concentration of FA in Bap-exposed mouse serum was also reduced (Fig. 2B). We further investigated the impact of FOLR1 overexpression on FA levels and found that the intracellular concentration of FA increased before 12 h and then decreased, while extracellular FA reduced consistently from 6 h. Compared to the FOLR1 overexpression group, the Vector control group showed a consistent decrease in extracellular FA levels and an increase in intracellular FA levels after 12 h (Fig. 2C). To further explore the role of FA in Bap-mediated suppression of VSV replication, we inhibited the expression of VSV-G mRNA and protein in vitro through Bap stimulation, pFOLR1 over-expression, and FA starvation culture, respectively. We found that the expression of VSV-G was rescued by FA supplement (Fig. 2D–I). Furthermore, the suppression of VSV replication induced by Bap exposure was also reversed by FA supplementation in the lung tissues of mice as shown in Fig. 2J and Fig. 2K.

We, therefore, speculated that Bap-induced upregulation of FOLR1 led to the condition of FA deficiency, which activated cellular antiviral mechanisms to suppress VSV replication.

3.3. FOLR1-mediated FA deficiency inhibited VSV replication via upregulating APOBEC3 expression

A previous study has demonstrated that the family of APOBEC3s is involved in antiviral innate immunity (Cheng et al., 2019). Most of APOBEC3 proteins act on DNA viruses or the DNA intermediate of retroviruses while its impact on RNA viruses is less studied. In our study, multiple Apobec3 genes were significantly upregulated in Bap-stimulated-HeLa cells, such as Apobec3a, Apobec3b, Apobec3c, Apobec3f, Apobec3g, and Apobec3h. Notably, Apobec3b was upregulated by Bap stimulation (≥ 9-fold) (Fig. 3A and Supplementary Fig. S2B). The mouse genome encodes a single Apobec3 gene (Salas-Briceno et al., 2020), and APOBEC3 mRNA was also upregulated in lung tissues of Bap-exposed mice (Fig. 3B). We found that the antiviral effect of Bap was partly abolished in HeLa cells with Apobec3 family gene (Apobec3a-h) knock-down, and APOBEC3B had the largest contribution to reducing VSV replication (Fig. 3C–D, and Supplementary Fig. S2C). APOBEC3B has been reported to suppress the replication of several viruses, and evolve endogenous double-stranded RNA (dsRNA) structures (Stavrou and Ross, 2015; Sadeghpour et al., 2021). dsRNA is one of the pivotal intermediate in RNA virus replication (Peisley and Hur, 2013), therefore, we focused on APOBEC3B. To confirm the positive correlation between the condition of FA deficiency and APOBEC3B activation, we established three models of FA deficiency: first, we measured the positive correlation between APOBEC3B expression and FA deficiency, and APOBEC3B expression was significantly upregulated in the model of Bap-mediated FA deficiency in vitro (Fig. 3E). Secondly, APOBEC3B was also highly expressed in the model of FOLR1 overexpression-mediated FA deficiency in vitro (Fig. 3F). Finally, in the cell cultured with FA-free culture medium (FA starvation), the production of APOBEC3B was significantly upregulated (Fig. 3G), which could be reduced by FA supplementation in a dose dependent manner (Fig. 3H). Furthermore, FA supplementation reduced the expression of APOBEC3B caused by...
Bap-stimulation, FOLR1 overexpression, and FA starvation culture (Fig. 3I). As expected, APOBEC3B knock-down rescued the expression of VSV-G protein and VSV RNA (Fig. 3J–K). These observations suggested an important role of FA metabolism in modulating APOBEC3B expression to support the host antiviral response.

3.4. Methotrexate (MTX) inhibited RNA viruses by targeting APOBEC3B

MTX is an anti-folate agent that competitively inhibits FA metabolism, and is widely used in the treatment of tumors and arthritis (Chan and Cronstein, 2010). We evaluated the antiviral activity of MTX and elucidated its mechanism both in vitro and in vivo. We found that MTX potently inhibited the replication of VSV both in vitro and in vivo as determined by the expression of VSV-G mRNA, VSV viral RNA and VSV-G protein (Fig. 4A–C) in HeLa cells and in lung tissues of mice (Fig. 4D and 4E). The expression of APOBEC3B protein and mRNA were both significantly upregulated by MTX treatment in HeLa cells with or without viral infection (Fig. 4F and 4G). Additionally, APOBEC3B was upregulated in a dose dependent manner in the presence of MTX (Fig. 4H and 4I). MTX treatment also upregulated APOBEC3B mRNA expression in lung tissues of mice (Fig. 4J). Furthermore, the expression of VSV-G was rescued by APOBEC3B knock-down (Fig. 4Ka and 4L). Interestingly, we found that MTX treatment significantly reduced the mRNA expression of other RNA viruses, such as RSV, EV71, CA16 and SFSTV without causing significant cytotoxicity in HeLa cells (Supplementary Figs. S2D and S2E). In summary, our data suggest that MTX-induced deficiency of FA is required for the activation of APOBEC3B-mediated antiviral activity and FA metabolism is not only critical for the replication of VSV, but also for other RNA viruses (Fig. 5).
Environmental pollutants (e.g., PM$_{2.5}$, NOx, etc.) have been reported to impact on various biological targets, and to result in diverse pathological consequences (Li et al., 2020; Li et al., 2021). However, in addition to their toxic effects, some pollutants were reported to influence different stages of the viral life cycles, suggesting a potential role in the development and progress of viral infectious diseases (Mishra et al., 2020). In the current study, we identified Bap, a pollutant widely present in human daily life, activated the axis of FOLR1/FA deficiency/APOBEC3B, which played a critical role in VSV replication.

4. Discussion

Type I interferons (IFNs) (Gokhale et al., 2021) and aromatic hydrocarbon receptors (AhR) are known to play important roles in antiviral innate immunity (Yamada et al., 2016). Our results indicated that neither AhR nor IFNs signaling pathways were involved in Bap-mediated inhibition of VSV (Supplementary Figs. S3A–F), suggesting a distinct mechanism of Bap-mediated inhibition of VSV. Subsequently, our data illustrated that the condition of FA deficiency, mediated by Bap-induced expression of FOLR1, contributed to the reduction of VSV replication both in vitro and in vivo. FOLR1 is a transmembrane transporter protein that participates in nucleic acid synthesis, methylation, and repair (Cheung et al., 2016). The transient replication of viral genome needs a...
large amount of purine substrate and methylation modifications from the host, which is facilitated by the availability of FA (White et al., 2011; Konan and Sanchez-Felipe, 2014). For instance, SARS-CoV-2 has the capacity to co-opt FA-mediated 1C metabolism with the host to build a novel RNA replication factory (Zhang et al., 2021); while FA deficiency has been reported to perturb virus maturation after an initial HPV infection and suppressed viral replication (Pathak et al., 2014). A previous report have shown that the activation of the DNA repair mechanism was associated with Bap exposure (Allmann et al., 2020). Another study demonstrated FOLR1 was elevated to increase FA uptake in order to...
Fig. 4. MTX exhibits a broad antiviral activity via the upregulation of APOBEC3B. A, B HeLa cells were pretreated with MTX from 0.5 μmol/L to 10 μmol/L for 2 h, and then they were infected with VSV (MOI = 1) for 24 h. The expression of VSV-G mRNA, VSV viral RNA and VSV-G protein were measured by qPCR and Western blot, respectively. C HeLa cells were pretreated with MTX 1 μmol/L for 2 h, and then they were infected with VSV (MOI = 1) for 24 h. VSV viral RNA was detected by qPCR assay. D 10 female mice were divided equally into two groups, and then the infection of VSV (5 x 10^4 PFU) following the model establishment of MTX (0.5 μg/mouse, n = 5) or equal doses of DMSO (n = 5) treatment in mice. E The expression of VSV-G mRNA and VSV viral RNA in the lung tissues of mice were detected 48 h post infection by qPCR analysis. F, G HeLa cells were pretreated with MTX 1 μmol/L, and then they were infected with VSV (MOI = 1) for 24 h. Western blot and qPCR were used to detect APOBEC3B and GAPDH expression. H, I The expression of APOBEC3 mRNA and protein in HeLa cells were measured by qPCR and Western blot, respectively. J The expression of APOBEC3 mRNA in the lung tissues of mice were detected by qPCR analysis. K, L siAPOBEC3B knockdown or siNC HeLa cells were pretreated with MTX at 1 μmol/L for 2 h, and then they were infected with VSV (MOI = 1) for 24 h. The expressions of VSV-G and GAPDH mRNA and protein were measured by qPCR and Western blot. Data expressed as means ± SD (n = 3–5, *P < 0.05, **P < 0.01, ***P < 0.001, ‘ns’ means no significant difference).
repair DNA damage (Low, 1989). Our results suggest that Bap treatment induced DNA damage is associated with FOLR1 upregulation and FA deficiency. Based on above evidence, we concluded that the limited availability of FA in the cell culture was depleted by Bap treatment, which made the cells unable to support the replication of viruses and activated the cellular antiviral mechanisms.

There is limited information on the role of FA metabolism in the regulation of antiviral immune system. Thus, it was of great interest that FA deficiency activates APOBEC3 proteins expression. APOBEC3 proteins consist of seven cytidine deaminases (A to H), and are considered as innate defense factors in fighting against various viral infections (Green and Weitzman, 2019). The incorporation of APOBEC3 into viral particles can induce G-to-A hypermutations in the genome of virus, serving as a cellular antiviral mechanism against viruses such as hepatitis B virus (HBV) (Chen et al., 2018), and human immunodeficiency virus (HIV) (Bandarra et al., 2021). In addition, APOBEC3B can efficiently recognize and dC to cause viral nucleotide damage (Diamond et al., 2019; Granadillo Rodríguez et al., 2020). Cellular APOBEC3B can damage Epstein-Barr virus genomic integrity (Shaban et al., 2022). Another study showed that APOBEC3B enzyme is partially localized to polyomavirus replication centers to suppress viral replication (Verhalen et al., 2016). Recently, it has been reported that APOBEC3B could interact with HBV core protein in an RNA-dependent manner and edit HBV DNAs during reverse transcription (Chen et al., 2018). Furthermore, APOBEC3B degrades HBV cccDNA in the nucleus (Lucifora et al., 2014).

In our study, we firstly found that the increased expression of APOBEC3B was associated with FA deficiency, which was consistent with a previous observation that FA deficiency was associated with the APOBEC3 family (Söderström et al., 2013). This is reasonable because FA deficiency (Duthie, 2011) and APOBEC3B are both important factors involving in DNA damage and/or repair system (Conner and Shaik, 2020). Furthermore, MTX, as one of the anti-folates, upregulated the expression of APOBEC3B in vitro and the expression of APOBEC3s in vivo, suggesting that FA-APOBEC3B is a key axis involved in the antiviral effects.

5. Conclusions

In summary, our present work elaborated the roles of FOLR1-mediated FA metabolism in the replication of RNA viruses. Mechanistically, our data showed that FOLR1-mediated FA deficiency reduced the replication of RNA viruses via upregulating APOBEC3B expression, which will shed light on the relationship between FA metabolism and
viral infections, and provide an insight into potential antiviral drug development.

Data availability

The RNA-seq datasets generated and analyzed during the current study are available in the National Center for Biotechnology Information Gene Expression Omnibus database with accession number GSE191225. And other datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics statement

All animal experimental protocols were approved by the Nanjing University Animal Care Committee and followed the ‘Guide for the Care and Use of Laboratory Animals’ published by the Chinese National Institutes of Health. The research protocols were conducted in accordance with the animal behavioral guidelines, using approved protocols from the institutional animal care committee.

Author contributions

Jing Wu, Deyan Chen, Yajing Han, Ruining Lyu: conceptualization; data curation, validation and analysis; software; writing of original draft; Fang Zhang, Na Jiang, Hongji Tao, Qiao You, Rui Zhang, Meng Yuan: funding acquisition and resources. All authors read the article and approved the submitted version.

Conflict of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.virs.2023.04.001.

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