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RSEARCH ARTICLE

M2 pyruvate kinase enhances HIV-1 transcription from its long terminal repeat

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Abstract Both thymocytes and tumor cells express M2 type isoenzyme of pyruvate kinase (M2PK), which is different from R type isoenzyme of pyruvate kinase (RPK) that is expressed in erythrocytes. In this report, the effect of RPK and M2PK on the transcription of human immunodeficiency virus type 1 (HIV-1) was tested. The results indicated that M2PK could enhance HIV-1 transcription from its long terminal repeat (LTR) promoter, while RPK did not have such an effect. Specific down-regulation of M2PK could inhibit HIV-1 transcription from its LTR region. Furthermore, it was found that the C terminal region of M2PK is responsible for this effect. Collectively, the cellular factor M2PK that is expressed in thymocytes could facilitate the transcription of HIV-1.

Keywords Human immunodeficiency virus type 1 (HIV-1), transcription, M2 type isoenzyme of pyruvate kinase (M2PK), R type isoenzyme of pyruvate kinase (RPK), nuclear factor κB (NFκB), long terminal repeat (LTR)

1 Introduction

Gene expression and viral replication of human immunodeficiency virus type 1 (HIV-1) is controlled by complex regulatory mechanisms involving transcriptional as well as posttranscriptional events (Cann and Karn, 1989; Cullen and Greene, 1989). In particular, once the HIV-1 provirus is integrated into the host cell genome, transcription of the HIV-1 provirus is then regulated by an interplay between a combination of distinct viral and cellular transcription factors with binding sites present in the HIV-1 long terminal repeat (LTR) (Garcia and Gaynor, 1994; Kingsman and Kingsman, 1996; Pereira et al., 2000). The LTR is divided into four functional regions: transactivation response element (TAR), core, enhancer, and modulatory elements (Pereira et al., 2000; Rohr et al., 2003). To date, a large number of studies on cellular factors binding to these regions of the HIV-1 LTR have been reported. The viral transactivator–Tat specifically binds to TAR RNA. The core region encompasses the initiator (Inr), the TATA box, and three Sp1-binding sites. The enhancer region of HIV-1 LTR contains two adjacent nuclear factor κB (NFκB)-binding sites and the modulatory region harbors binding sites for numerous transcription factors, such as c-Myb, nuclear factor of activated T cells (NF-AT), upstream stimulation factor (USF), and activator protein-1 (AP1) (Rohr et al., 2003).

Pyruvate kinase (PK, ATP: pyruvate 2-*O*-phosphotransferase, EC 2.7.1.40), a key enzyme in the glycolytic pathway, has four tissue-specific isoenzymes in mammals: L-type (hepatic) and R-type (erythrocytic), encoded by the *PK-LR* gene under the control of two tissue-specific promoters, and M1-(muscle, heart, and brain) and M2-(fetal, tumor, and most adult tissues) types encoded by the *PK-M* gene by alternative mRNA splicing (Noguchi et al., 1986; Noguchi et al., 1987). HIV-1 mainly infects a variety of immune cells such as CD4 + T cells, macrophages, and microglial cells and all of these cells stem from thymus, which mainly express M2 type isoenzyme of pyruvate kinase (M2PK) (Netzker et al., 1992). Although HIV-1 is transmitted by exposure to infected blood, it does not infect erythrocytes that express R type isoenzyme of pyruvate kinase (RPK). Moreover, most studies describing how cis- and trans-acting elements regulate LTR-driven transcription are based on the observation in various tumor cells such as HeLa, 293T, NIH3T3, and Jurkat cells (Pereira et al., 2000; Rohr et al., 2003). No matter which kind of tissue these tumor cells are derived from, they all express M2PK. Although M2PK is an important enzyme in the glycolytic pathway, numerous evidences indicated that it may also be involved in carcinogenesis, apoptosis, development, cellular growth, and immune and inflammatory responses (Presek et al., 1988; Eigenbrodt et al., 1992; Zwerschke et al., 1999; Stetak et al., 2007; Shimada et al., 2008; Wu et al., 2008b). Since there exists a correlation between HIV

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replication and M2PK-expressing cells, we try to figure out whether and how M2PK is involved in HIV-1 replication.

In this report, we tested the effect of erythrocyte-specific RPK and M2PK on the transcription of HIV-1, respectively. Our data demonstrated that M2PK could enhance HIV-1 transcription, while RPK did not have such an effect. Furthermore, specific down-regulation of M2PK could inhibit HIV-1 transcription from its LTR region.

2 Materials and methods

2.1 Plasmids

Plasmid pNL4-3lucR-E- containing a firefly luciferase reporter gene and deficient for envelope synthesis was obtained through the National Institutes of Health AIDS Reagent Repository (Connor et al., 1995; Modem et al., 2005). Sequences encoding the full-length human M2PK (aa 1–531), L type isoenzyme of pyruvate kinase (LPK) (aa 1–543), and the truncated M2PK (CM2PK) (aa 333–531) were amplified by PCR using cDNA from Huh7 cells and then cloned into plasmid pCI-Neo (Promega) with an ATG-HA-tag at the N terminus as described in our previous work (Wu et al., 2008b). Since RPK and LPK were transcribed from the same gene by using different promoters, the full-length fragment of RPK was obtained by PCR using LPK as a template to add the missing N terminus and then cloned into the pCI-Neo vector. The luciferase reporter plasmid pNFκB-luc, p65 and MAVS/VISA expressing constructs were kindly provided by Dr. Shu (Xu et al., 2004; Tian et al., 2007). Plasmid expressing HIV-1 Tat was obtained by inserting Tat fragment into pCI-Neo vector. HIV-1 LTR DNA sequence was amplified by PCR from pNL4-3lucR-E- and inserted into the pGL3.0 vector (Promega) to obtain wild-type HIV-1 LTR-luc. LTR mutant (LTRDκB-luc) was generated by site-directed mutagenesis at both conserved NFκB sites (nt-105 and-78) as described in our previous work (Wu et al., 2008a).

2.2 Cell culture and transfection

Human 293T cells were obtained from China Center for Type Culture Collection (CCTCC), Wuhan, China. Cells were cultured and maintained in standard Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), penicillin at 100 U/mL, and streptomycin at 100 μg/mL in 5% CO_2 at 37°C. Transfection of cells was carried out with Lipofectamine 2000 reagent (Invitrogen) by following the manufacturer's instructions.

2.3 RNA interference

By using the small interfering RNA (siRNA) Finder (Ambion, http://www.ambion.com/techlib/misc/siRNA_finder.html), the M2PK-targeting sequence 5'-TTCCACCACCTTGCAGATGTT-3' was selected from the human M2PK cDNA sequence (GenBank accession number NM-182471) and it did not show any homology to other known genes. Sense and antisense DNA oligonucleotides encoding the siRNA against M2PK RNA were synthesized, annealed, and cloned into the pSilencer1.0 U6 siRNA expression vector (Ambion). The small hairpin RNA (shRNA) plasmid was designated as shM2PK. Control plasmids for shRNAs LucR, Daxx, and CT-4 were described previously (Li et al., 2005).

2.4 Western blot analysis

The transfected cells were harvested and rinsed twice with phosphate-buffered saline (PBS), pH 7.4. Cell extracts were prepared by incubating cells in lysis buffer (50 mmol/L Tris [pH 8.0], 120 mmol/L NaCl, 0.5% NP-40) containing protease inhibitors (2 μg of aprotinin/mL, 1 μg of leupeptin/mL, and 2.5 mmol/L phenylmethylsulfonylfluoride) and cleared by centrifugation at 12000 r/min at 4°C, as described previously (Wu et al., 2008b). Protein concentrations were determined by a Bio-Rad protein assay, and 20 μg of total protein was loaded onto 10% sodium dodecyl sulfate (SDS)-polyacrylamide gels and then transferred to a polyvinylidene difluoride membrane (PVDF; Invitrogen). After blocking with 5% non-fat dried milk in PBS/Tween 20 buffer, the blots were incubated with the first antibody for 2 h at room temperature and subsequently incubated for 40 min with the second antibody. Then, the membranes were incubated with the enhanced chemiluminescence solution (Pierce) for 5 min and exposed to X-ray film (Kodak). The antibody against M2PK (Protein Tech Group) was used at a dilution of 1:1000. The antibody against β-actin and HA were purchased from Sigma and used at a dilution of 1:2000.

2.5 Luciferase reporter assay

The 293 T cells growing in 96-well plates were transfected with the indicated test plasmids and luciferase reporter constructs along with a pRL-CMV plasmid (Promega) as an internal control by using Lipofectamine 2000 Reagent (Invitrogen) according to manufacturer's instructions. Forty-eight hours post-transfection, cells were lysed and the cell lysates were used for the measurement of firefly and Renilla luciferase activities. Firefly luciferase activity was normalized relative to the Renilla luciferase activity to control for the difference in transfection efficiency.

2.6 *In vivo* fluorescence analysis

A pEGFP-C1 reporter plasmid (1 μg) and shRNA expression plasmid (3 μg) were cotransfected into 293T cells by use of Lipofectamine 2000 Reagent, as described above. The fluorescence in living cells was visualized at 50 h

posttransfection by conventional fluorescence microscopy. For green fluorescent protein (GFP) fluorescence detection, a fluorescein isothiocyanate filter was used.

2.7 Statistical analysis

All experiments were repeated at least three times. Results are expressed as mean±standard error. Statistical analysis was performed by using Microsoft Excel and OriginPro 7.5 software. *P* values were determined by using paired *t* test. Differences were considered to be significant when $P < 0.05$.

3 Results

3.1 Expression of M2PK enhances the HIV-1 gene expression in an isoenzyme-specific manner

To address the effect of different PK isoenzymes on the HIV-1 transcription, a comparative dose-response analysis was performed. The 293T cells were transfected with an empty vector (150 ng/well) or indicated amount of M2PK or RPK expression plasmid along with the pNL4-3lucR-E- construct (50 ng/well) and pRL-CMV (20 ng/well) and luciferase assay was performed 48 h post-transfection. The total amount of transfected DNA was kept constant by adding certain amount of empty vector. The results indicated that transient expression of M2PK could significantly increase the reporter gene expression from HIV-1 replicon in a dose-dependent manner whereas RPK could not (Fig. 1). This result implied that M2PK probably plays a role in the regulation of HIV-1 replication or transcription.

3.2 HIV-1 gene expression is specifically inhibited by depleting M2PK with RNA interference

To further prove the involvement of M2PK in HIV-1 replication or transcription, we employed an shRNA (named as shM2PK) to specifically down-regulate the expression of endogenous M2PK, and then test its specific inhibition on the HIV-1 gene expression. The shRNA CT-4 which targets cyclin T1 had already been shown capable of efficiently inhibiting the HIV-1 transcription in our previous work (Li et al., 2005) and thus was used as a positive control. The 293T cells were separately transfected with M2PK, RPK, empty vector with or without shM2PK, and Western blot analysis was performed on total cell lysates 48 h post-transfection using anti-HA or anti-β-actin antibodies. As shown in Fig. 2A, the expression of M2PK, but not RPK, in shM2PK-transfected cells was significantly down-regulated.

To determine whether this shM2PK could inhibit gene expression of HIV-1, the 293T cells were transfected with mock, shM2PK or shRNA CT-4 (150 ng/well) along with pNL4-3lucR-E- (50 ng/well) and pRL-CMV (20 ng/well), and the luciferase activity was assayed 48 h post-transfection. The results showed that both shM2PK and shRNA CT-4 reduced the luciferase activity significantly in comparison with the mock control. The inhibitory efficiency of shM2PK was similar to that of shRNA CT-4 (Fig. 2B).

We next tested whether the inhibition of HIV-1 gene

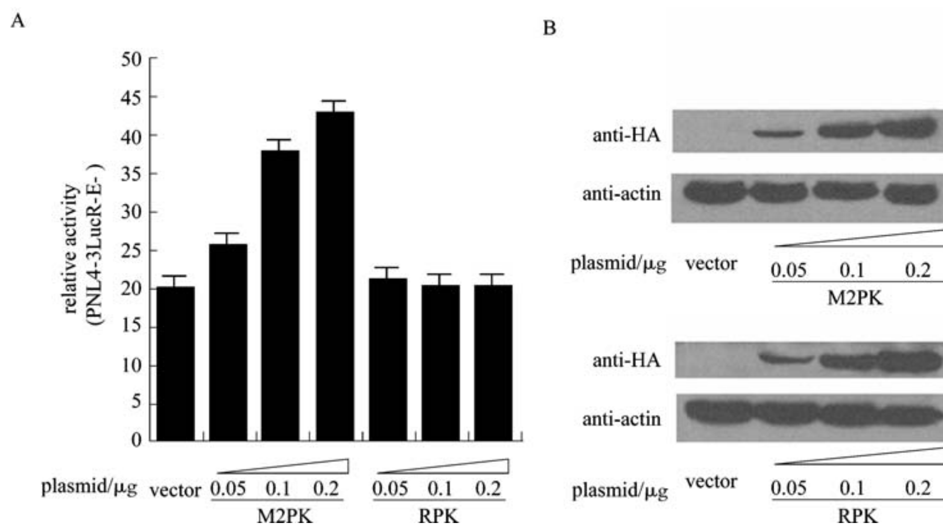

Fig. 1 Exogenous expression of M2PK enhances the HIV-1 transcription in an isoenzyme-specific manner. (A) Dose-response analysis of the effect of thymocyte-specific M2PK and erythrocyte-specific RPK on HIV-1 transcription. (B) Corresponding M2PK and RPK expression was determined by Western blot analysis, using anti-HA or anti-β-actin antibodies, respectively. M2PK: M2 type isoenzyme of pyruvate kinase; RPK: R type isoenzyme of pyruvate kinase; HA: a tag at the N terminus (described in our previous work, Wu et al., 2008b).

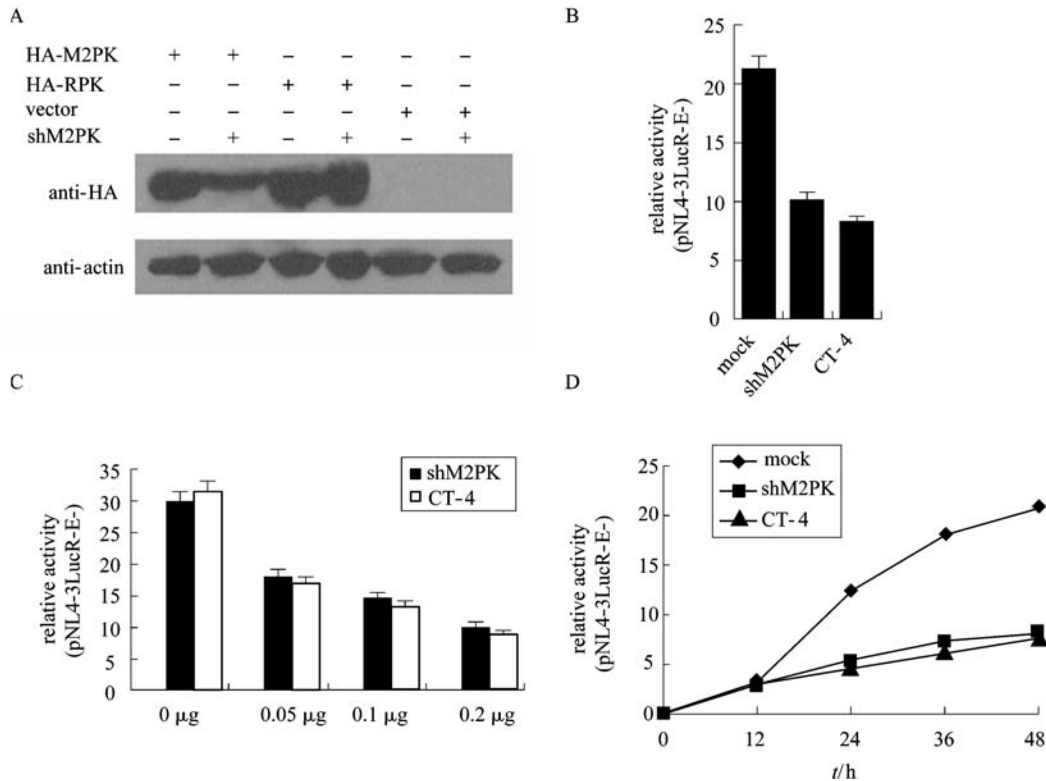

Fig. 2 Specific inhibition of the HIV-1 transcription by depleting M2PK with small interference RNAs. (A) Western blot analysis for 293T cells transfected with M2PK, RPK, empty vector with or without shM2PK. (B) Luciferase activity assay for 293T cells transfected with mock, shM2PK or shRNA CT-4 along with pNL4-3lucR-E-and pRL-CMV. (C) Dose-dependent inhibition of HIV-1 gene expression by shM2PK or shRNA CT-4. (D) Time-dependent inhibition of HIV-1 gene expression by shM2PK or shRNA CT-4. M2PK: M2 type isoenzyme of pyruvate kinase; RPK: R type isoenzyme of pyruvate kinase; shRNA: small hairpin RNA; shM2PK: shRNA plasmid to M2PK.

expression by shM2PK was dose- and time-dependent. The 293T cells were co-transfected with 20 ng pNL4-3lucR-E- and indicated dose of shM2PK or shRNA CT-4 (0, 0.05, 0.1, 0.2 μg) along with 20 ng pRL-CMV per well in 96-well plates, and 48 hours after transfection, luciferase activity assay was performed. As shown in Fig. 2C, increase in the amount of the shM2PK-expressing plasmid from 0.05 to 0.2 μg per well down-regulated gene expression level of HIV-1 in a gradient manner, indicating that the inhibition took place in a dose-dependent manner. Moreover, HIV-1 transcription was detected at different time points post transfection and the results showed (Fig. 2D) that down-regulation of M2PK could efficiently inhibit HIV-1 transcription in a prolonged period until at least 48 h post transfection. Taken together, these data indicated that M2PK may be involved in the regulation of HIV-1 gene expression in a dose- and time-dependent manner.

3.3 Down-regulation of M2PK could inhibit the transcription driven by HIV-1 LTR promoter

To reveal whether the involvement of M2PK took place at the transcriptional stage from HIV-1 LTR region, we generated a luciferase reporter construct where the reporter expression was under the control of the HIV-1 LTR promoter. As shown in Fig. 3A, transient transfection of M2PK shRNA construct shM2PK led to significant inhibition of the reporter gene expression from HIV-1 LTR in 293T cells. This result implied that M2PK was involved in the transcription of HIV-1.

Among all these distinct cellular and viral transcription factors known to bind to the HIV-1 LTR, the nuclear transcription factor NFκB is a key regulator of HIV-1 gene expression in cells latently infected with this virus (Nabel and Baltimore, 1987; Phares et al., 1992; Roulston et al., 1995; Mallardo et al., 1996; Montano et al., 1996). To investigate the possible involvement of NFκB in the enhancement of HIV-1 transcription by M2PK, the 293T cells were transfected with individual shRNA-expressing constructs along with p65 and an NFκB luciferase reporter construct (NFκB-luc). Here shRNA CT-4 was still used as a positive control since the activation of transcription by p65 also depends on P-TEFb, which was a heterodimer composed of cyclin-dependent kinase 9 (CDK9) and cyclin T1 (Barboric et al., 2001). The results showed that both shM2PK and CT-4 could effectively inhibit p65 induced NFκB activation, implying M2PK also played a

potential role in the activation of NFκB (Fig. 3B). In a parallel experiment, we also tested the effect of these shRNAs on an NFκB binding site deletion construct of HIV-1 LTR (LTRDκB-luc) and the result indicated that both shM2PK and shRNA CT-4 could inhibit the luciferase activity when compared with controls (Fig. 3C). These data showed that the enhancement effect of M2PK on HIV-1 transcription was not only resulted from the regulation of basic transcription of HIV-1 LTR, but also caused by regulation of the enhancer region of HIV-1 LTR.

Then we investigated whether M2PK shRNA (or the down-regulation of M2PK) had some toxic effects on normal cell viability, thus resulting in down-regulation of general gene expression. For this purpose, the 293T cells were co-transfected with 0.2 μg pEGFP-C1 reporter plasmid together with 0.2 μg shM2PK, LucR shRNA, and Daxx shRNA, respectively. GFP reporter gene expression and cellular morphology and density were then monitored at 50 h post-transfection by fluorescence imaging under a microscope. The results showed that there was no significant difference in green fluorescence expression in mock, shM2PK, or LucR shRNA-transfected cells compared to apoptosis-inducing Daxx shRNA, indicating that down-regulation of M2PK by shM2PK was not toxic to the cells (Fig. 3D). These results showed that the inhibitory effect of M2PK shRNA (shM2PK) on HIV-1 transcription with or without NFκB-binding sites in the LTR region did not result from a toxic effect or influence of cell viability.

Collectively, the above results suggested that the M2PK could enhance transcription of HIV-1 via regulation of multiple signaling pathways. Although the exact molecular mechanism was not known, it was most probably that M2PK could regulate some general transcription factors essential for basic transcription.

3.4 The C-terminal region of M2PK is responsible for the enhancement effect on HIV-1 transcription

As shown above, M2PK possessed the capability to enhance HIV-1 transcription but RPK did not. Although M2PK and RPK are encoded by different genes, they share

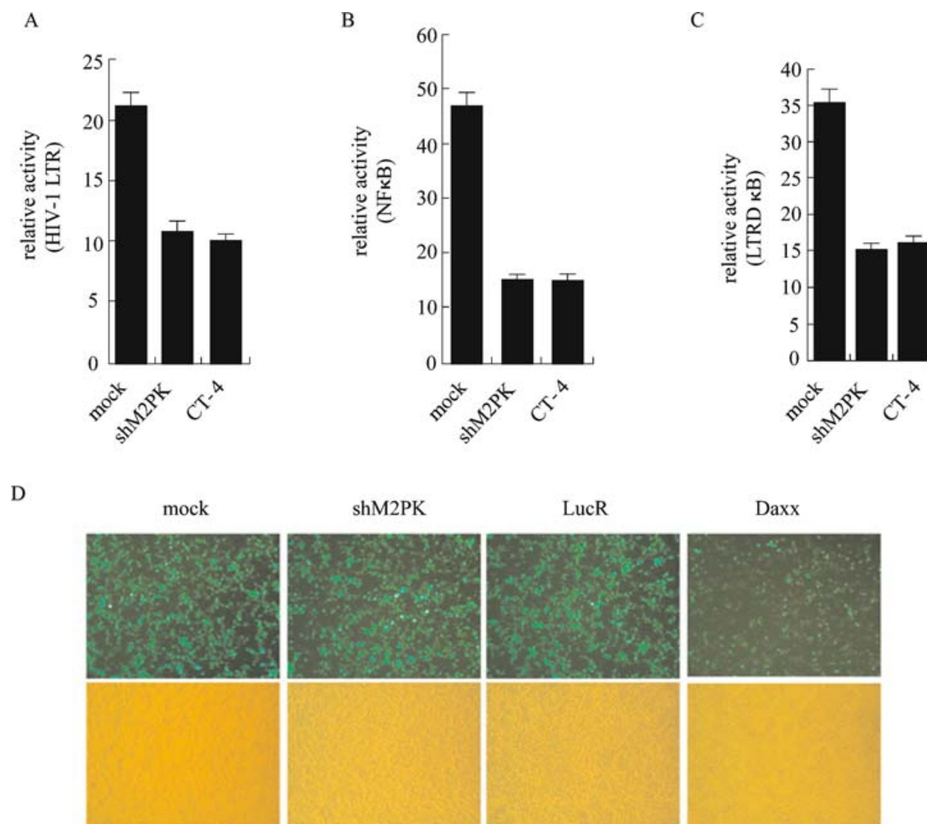

Fig. 3 Down-regulation of M2PK could inhibit the transcription driven by HIV-1 LTR. The 293T cells were transfected with mock, shM2PK or shRNA CT-4 (150 ng/well) along with pRL-CMV (20 ng/well) and indicated plasmids: (A) HIV-1 LTR luciferase reporter construct (50 ng/well), (B) NFκB luciferase reporter construct (20 ng/well) and p65 (20 ng/well), or (C) LTRDκB luciferase reporter construct (50 ng/well). (D) Cell viability analysis was determined by *in vivo* fluorescence expression in 293T cells co-transfected with pEGFP-C1 plasmid and of shM2PK, shRNA LucR, or shRNA Daxx plasmid, respectively. M2PK: M2 type isoenzyme of pyruvate kinase; LTR: long terminal repeat; NFκB: nuclear factor κB; LTRDκB: NFκB binding site deletion. RPK: R type isoenzyme of pyruvate kinase; shRNA: small hairpin RNA; shM2PK: shRNA plasmid to M2PK.

many features: the same pyruvate kinase activity, 71% amino acids identity, and very similar three-dimensional structures. The most significant difference between them lies in their regulatory domains, which are located at the C terminal region of both proteins. To figure out whether the C-terminal region is responsible for the enhancement effect of M2PK on HIV-1 transcription, we constructed a plasmid expressing the C terminal region of M2PK (CM2PK). The 293T cells were transfected with mock, empty vector, M2PK, CM2PK or RPK (150 ng/well) along with pNL4-3lucR-E- (50 ng/well) and pRL-CMV (20 ng/well), and after 48 hours of transfection, luciferase activity assay was performed. The results showed that both M2PK and CM2PK could enhance the transcription of HIV-1 as indicated by the reporter activity 48 h post transfection, while RPK could not (Fig. 4B). This result indicated that the C-terminal region of M2PK is responsible for the enhancement effect on HIV-1 transcription.

To further confirm such an effect, the 293T cells were transfected with an empty vector or M2PK expressing plasmid (100 ng/well) along with three different NFκB activator (Tat, p65 or MAVS/VISA) expressing plasmids (50 ng/well), an NFκB luciferase reporter construct (50 ng/well) and pRL-CMV (20 ng/well). As shown in Fig. 4C, M2PK could enhance the activation of NFκB induced either by Tat, p65 or MAVS/VISA.

To investigate whether the C-terminal region of M2PK is also responsible for the enhancement effect on NFκB activation, the 293T cells were transfected with an empty vector, M2PK, CM2PK or RPK (150 ng/well) along with p65 and NFκB-luc (20 ng/well), and pRL-CMV (20 ng/well), and luciferase assay was performed 48 hours post-transfection. The result showed that both M2PK and CM2PK could enhance the activation of NFκB, while RPK failed to do so (Fig. 4D). These results suggested that the C-terminal regulatory domain of M2PK was also involved in the NFκB-mediated activation. Taken together, these data indicated that the C-terminal regulatory domain of M2PK may be involved in regulation of general transcription factors so that it could influence the transcription efficiency of different genes.

4 Discussion

HIV-1 mainly infects a variety of immune cells such as CD4 + T cells, macrophages, and microglial cells and all of these cells are derived from thymus, which mainly

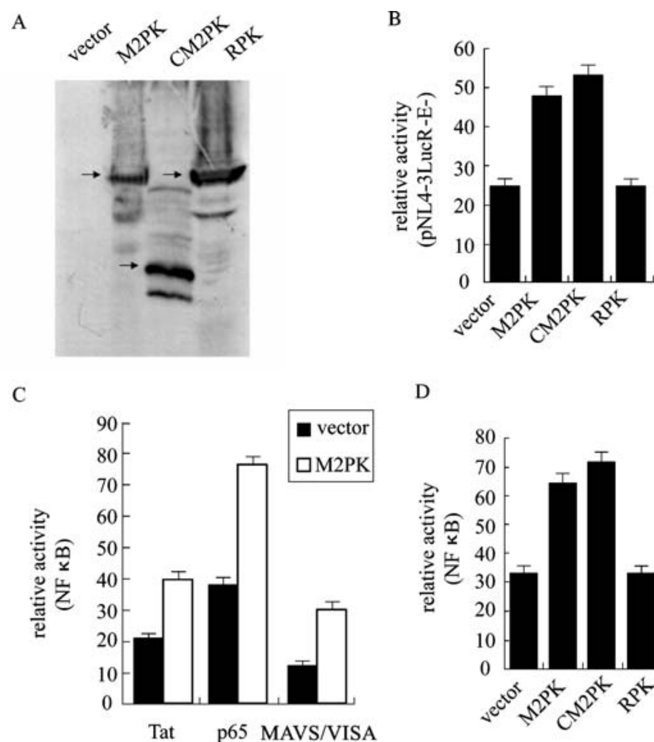

Fig. 4 The C-terminal region of M2PK is responsible for the enhancement effect on HIV-1 transcription. (A) Western blot assay for exogenous expression of M2PK, CM2PK and RPK by using anti-HA antibodies. (B) Luciferase activity assay for 293T cells transfected with empty vector, M2PK, CM2PK or RPK along with pNL4-3lucR-E- and pRL-CMV. (C) Luciferase activity assay for 293T cells transfected with an empty vector or M2PK expressing plasmid along with Tat, p65 or MAVS/VISA expressing plasmids, an NFκB luciferase reporter construct and pRL-CMV. (D) Luciferase activity assay for 293T cells transfected with an empty vector, M2PK, CM2PK or RPK along with an NFκB luciferase reporter construct, p65 expressing plasmid, and pRL-CMV. CM2PK: C terminal region of M2PK; RPK: R type isoenzyme of pyruvate kinase; shRNA: small hairpin RNA; NFκB: nuclear factor κB; Tat, p65 or MAVS/VISA: three different NFκB activators.

express M2PK (Netzker et al., 1992). Although HIV is transmitted by exposure to infected blood, it does not infect erythrocytes, which express RPK. In this study, we found that M2PK could enhance the transcription of HIV-1, while RPK did not have such an effect. Further study showed that knockdown of M2PK expression by RNAi inhibited HIV-1 replication specifically and efficiently. These results implied that M2PK may play an important role in HIV-1 replication. To explore the mechanism of this effect, we tested the effect of M2PK on the transcription of HIV-1 LTR. We observed that specific down-regulation of M2PK could inhibit the basic expression level of HIV-1 LTR and decrease the activation of NFκB pathway without cell toxicity. Finally, we found that overexpression of M2PK could enhance the activation of NFκB pathway and its C terminus was responsible for this effect.

As pyruvate kinase is an important enzyme involved in the glycolytic pathways, we tried to answer whether the effect of M2PK on HIV-1 transcription is due to some general functions of pyruvate kinase. First, M2PK but not RPK could enhance HIV-1 transcription (Fig. 1A). Second, the regulatory domain of M2PK that does not possess the enzymatic activity acted as the effective domain of M2PK (Fig. 4B). These two sets of data indicated that the effect of M2PK on HIV-1 transcription was not associated with the enzymatic activity of pyruvate kinase. Furthermore, when endogenous M2PK was knocked down, the transcription from HIV-1 LTR was reduced while GFP expression and cell viability were not obviously influenced. Taken together, the stimulatory effect of M2PK on HIV-1 transcription is most probably due to its regulatory function on HIV-1 transcription but not due to the enzymatic functions involved in the general metabolic pathways.

Pyruvate kinase is a rate-controlling glycolytic enzyme and catalyses the formation of pyruvate and ATP from phosphoenolpyruvate (PEP) and ADP. Expression of PK isoenzymes is tissue-specific and is developmentally regulated since individual tissues have their unique glycolytic metabolism requirement. The M2-type PK is considered to be the prototype isoenzyme since it is the only isoenzyme detected in early fetal tissues and progressively replaced by L-type PK (liver and kidney), M1-type PK (skeletal muscles, brain and heart) and R-type PK (erythrocytes) during development (Imamura and Tanaka, 1982) and re-expressed when cells de-differentiate to become malignant (Presek et al., 1988; Zwerschke et al., 1999). M2PK occurs in a highly active tetramer with a high affinity to its substrate PEP and a nearly inactive dimmer with a low affinity to PEP (Eigenbrodt et al., 1992; Mazurek et al., 1998; Mazurek et al., 2001; Mazurek et al., 2002). In tumor cells, the dimeric form is always predominant for a high rate of nucleic acid synthesis and has therefore turned out to be an ideal diagnostic marker for a number of cancers (Wechsel et al., 1999; Luftner et al., 2000; Schneider et al., 2000).

Presumably due to the central role of pyruvate kinase in the carcinogenic process, M2PK was shown to be directly targeted by some cellular factors and certain oncoproteins of virus. It is reported that the activated pp60v-src kinase can phosphorylate M2PK on a tyrosine residue, thus triggering a dimerization of M2-PK (Presek et al., 1988; Eigenbrodt et al., 1992). Human papilloma virus 16 oncoprotein E7, hepatitis C virus polymerase NS5B and the cytoplasmic promyelocytic leukemia (cPML) tumor suppressor protein can bind directly to M2PK (Zwerschke et al., 1999; Shimada et al., 2008; Wu et al., 2008b). Recently it was shown that nuclear translocation of M2PK is sufficient to induce cell death that is caspase-independent, isoenzyme-specific, and independent of its enzymatic activity (Stetak et al., 2007). In our report, we demonstrated that M2PK was important for the basic transcription of HIV-1 LTR and could enhance the activation of NFκB in an enzymatic activity independent manner. Although the function and mechanisms of M2PK in carcinogenic process remain elusive, all these researches imply that M2PK might play a potential role in the control of a variety of cellular processes, such as apoptosis, development, cellular growth, and immune and inflammatory responses.

To date, most of the studies describing how cis- and trans-acting elements regulate LTR-driven transcription are based on observations in various tumor cells such as HeLa, 293T, NIH3T3, and Jurkat cells (Pereira et al., 2000; Rohr et al., 2003). These tumor cells all express M2PK, the same type of PK isoenzyme as thymocytes possess. Therefore, based on our observation that M2PK could enhance HIV-1 replication in an isoenzyme-specific manner, we suppose that these tumor cells and thymocytes could provide a better milieu for HIV-1 replication when compared with other cell types.

In summary, our data demonstrate the enhancement effect of M2PK protein on transcription of HIV-1 and confirm the activation effect is in an isoenzyme-specific manner. Furthermore, we showed that M2PK was important for the basic transcription of HIV-1 LTR and could enhance the activation of NFκB in an enzymatic activity-independent manner. We conclude that the cellular factor M2PK specifically expressed in thymocytes facilitates the transcription of HIV-1.

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