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Silencing of HTLV-1 *gag* and *env* genes by small interfering RNAs in HEK 293 cells

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A B S T R A C T

Since the discovery of RNAi technology, several functional genomic and disease therapy studies have been conducted using this technique in the field of oncology and virology. RNAi-based antiviral therapies are being studied for the treatment of retroviruses such as HIV-1. These studies include the silencing of regulatory, infectivity and structural genes. The HTLV-1 structural genes are responsible for the synthesis of proteins involved in the entry, assembly and release of particles during viral infection. To examine the possibility of silencing HTLV-1 genes *gag* and *env* by RNA interference technology, these genes were cloned into reporter plasmids. These vectors expressed the target mRNAs fused to EGFP reporter genes. Three small interference RNAs (siRNAs) corresponding to *gag* and three corresponding to *env* were designed to analyze the effect of silencing by RNAi technology. The plasmids and siRNAs were co-transfected into HEK 293 cells. The results demonstrated that the expression of the HTLV-1 *gag* and *env* genes decreased significantly *in vitro*. Thus, siRNAs can be used to inhibit HTLV-1 structural genes in transformed cells, which could provide a tool for clarifying the roles of HTLV-1 structural genes, as well as a therapy for this infection.

1. Introduction

Human T-lymphotropic virus 1 (HTLV-1) is a member of delta retroviruses (Retroviridae family). It is the etiologic agent of adult T-cell leukemia-lymphoma (ATLL) (Ratner, 2004) and the neurologic disease HTLV-associated myelopathy/tropical spastic paraparesis (HAM/TSP) (Nagai and Osame, 2003). The HTLV-1 proviral genome is composed of *pol*, *gag* and *env* structural genes, and regulatory genes such as *tax* and *rex*, flanked by long terminal repeat (LTR) sequences at both ends (Seiki et al., 1983). The *pol* gene codes for the reverse transcriptase and integrase, and *gag* and *env* code for the structural proteins of HTLV-1. The mature matrix (MA), capsid (CA) and nucleocapsid (NC) proteins are produced after the cleavage of Gag precursor polyproteins by the viral protease. The HTLV-1 Env protein is derived from an envelope polyprotein precursor that is cleaved by cellular proteases, yielding a mature protein. This protein is composed of two subunits, a surface subunit (SU) and a transmembrane (TM) subunit (Le Blanc et al., 2001; Manel et al., 2005). Gag precursor polyproteins direct membrane targeting, which is required for viral assembly and release (Le Blanc et al., 2001). Previous studies found that HTLV-1 uses the glucose transporter GLUT-1 to infect the target cell, and this interaction involves also Env proteins (Manel et al., 2003). These features of HTLV-1 structural proteins make them important targets in studies directed toward the development of new therapeutic methods.

Observed initially in plants but described accurately in *Caenorhabditis elegans* (Fire et al., 1998), RNA interference (RNAi) refers to a cellular mechanism by which a double-stranded RNA (dsRNA) inhibits the expression of a specific gene. The dsRNAs are processed by the endonuclease Dicer into small interference RNAs (siRNAs), which are composed of approximately 21 nucleotides. These siRNAs are incorporated into the RNA-induced silencing complex (RISC) by the RISC-loading complex (RLC), and the sense strand of siRNA is removed. The RISC is guided by the siRNA antisense strand to the complementary target mRNA. Then, the mRNA is cleaved by the Ago2 protein. If partial complementarity occurs between the siRNA antisense strand and the target mRNA, translation is inhibited and there is no mRNA degradation (Kurreck, 2009). RNAi is involved in the inhibition of viruses and in the silencing of identical elements in plants, insects, fungi and nematodes (Waterhouse et al., 2001; Voinnet, 2001; Wilkins et al., 2005; Wang et al., 2006; Segers et al., 2007). When this genetic technique is used

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Table 1
Primer sequences used to amplify *gag* and *env* genes.

Primer	Gene	Sequence		
		Sequence to facilitate digestion	*Hind*III or *Bgl*II sites	*gag* or *env* gene sequence
P3GAG	*gag*	5′-**TTCTATCCC**	*AAGCTT*(H)	TTAAACCTCCCCCCCTATG-3′
P5GAG		5′-**TTCTATGGA**	*AGATCT*(B)	ATGGGCCAAATCTTTTCCC-3′
P3ENV	*env*	5′-**TTCTATCCC**	*AAGCTT*(H)	TTACAGGGATGACTCAGGG-3′
P5ENV		5′-**TTCTATGGA**	*AGATCT*(B)	ATGGGTAAGTTTCTCGCAA-3′

in mammals, dsRNAs elicit the innate antiviral immune response by inducing interferon-linked pathways (Rana, 2007). Furthermore, it has been found that the transfection of 21–23-nt synthetic siRNAs, with 2-nt 3′ overhangs, into mammalian cells effectively inhibits an endogenous gene in a sequence-specific manner (Elbashir et al., 2001; Caplen et al., 2001; Holen et al., 2002). Since 2001, when the first report on inhibition of human respiratory syncytial virus (RSV) using RNAi technology was published (Bitko and Barik, 2001), many other studies have been performed examining the suppression of viral infections (Ma et al., 2007; Haasnoot et al., 2007). They include the following: human immunodeficiency virus type 1 (HIV-1) (Novina et al., 2002; Hayafune et al., 2006; Naito et al., 2007), hepatitis C virus (HCV) (Krönke et al., 2004; Sakamoto et al., 2008), hepatitis B virus (HBV) (Wu et al., 2005a; Ying et al., 2007), severe acute respiratory syndrome coronavirus (Sars-CoV) (Shi et al., 2005; Wu et al., 2005b) and influenza A virus (IAV) (Zhou et al., 2007).

In the present study, siRNAs were used for inhibition of the expression of the HTLV-1 structural genes *gag* and *env*. Reporter systems were constructed that express viral target genes fused to the enhanced green fluorescent protein gene (EGFP). These genes were transformed into HEK 293 cells, and siRNAs were then introduced into cells expressing the structural proteins. The specific siRNAs corresponding to the HTLV-1 *gag* and *env* genes knocked down specifically these mRNAs, and inhibited the expression of Gag and Env proteins. These results may be important for the development of new gene therapy-based anti-HTLV-1 drugs.

2. Materials and methods

2.1. Construction of reporter systems for gag and env

Total RNA of HTLV-1-infected cells (MT-2, ECACC, Salisbury, Wiltshire, UK) was extracted by the TRIzol™ method and treated with DNAse I. Reverse transcription was performed using a high capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA). The entire *gag* and *env* genes were amplified by PCR. The primer sequences used are shown in Table 1. The amplified DNA fragments were digested with *Hind*III and *Bgl*II and inserted into the multi-cloning site of the pEGFP-C1 expression vector (Clontech, Mountain View, CA, USA) between the *Hind*III and *Bgl*II sites. These vectors express the EGFP reporter gene under cytomegalovirus (CMV) promoter control. In the resulting plasmids, pEGFP-Gag and pEGFP-Env, the EGFP gene was located upstream of the target genes (Fig. 1A).

2.2. Design and synthesis of small interfering RNAs

BLOCK-iT™ RNAi Designer software (Invitrogen, Carlsbad, CA, USA) was used to design the siRNAs corresponding to the *gag* and *env* genes (GenBank accession numbers X15951 and X56949 respectively). This algorithm performs a statistical analysis of the target sequence and correlates it with data collected from multiple validated siRNA oligo sets tested on different gene targets. Three siRNAs (siRNAs Gag1, Gag2 and Gag3) and one scrambled siRNA (siRNA Scr Gag) were designed for the *gag* gene, and three other siRNAs (siRNAs Env-1, Env-2 and Env-3) and one scrambled siRNA (siRNA Scr Env) were designed for the *env* gene (Fig. 1B and Table 2). One non-related siRNA was designed for both targets. All siRNA sequences were BLAST searched in the US National Center for Biotechnology Information (NCBI) against all human sequences deposited in the GenBank and RefSeq databases and no significant similarities with human genes were found. The siRNAs were resuspended in 1 ml of nuclease-free water for further use.

2.3. Transfection of HEK 293 cells

The human embryonic kidney cell line (HEK) 293 was cultured and maintained in Dulbecco's modified Eagle's medium (DMEM) (Gibco-BRL, Gaithersburg, MD, USA) containing 10% heat-inactivated fetal bovine serum (Gibco-BRL), supplemented with L-glutamine (1 mM), streptomycin (100 μg/ml) and penicillin (100 U/ml) (Gibco-BRL) at 37 °C in a 5% CO_2 incubator. Forty-eight

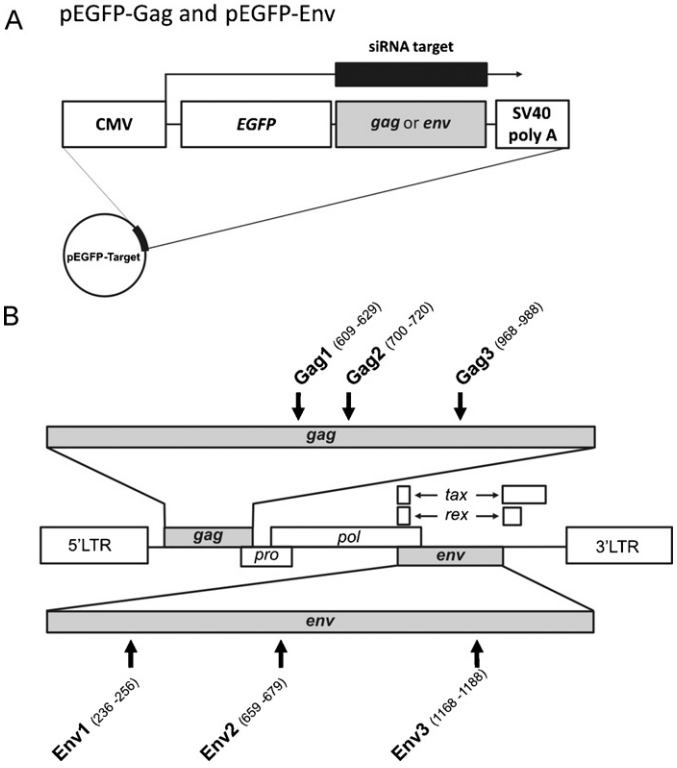

Fig. 1. Reporter plasmid and HTLV-1 genome targets. (A) Schematic illustration of the construction of the plasmids, pEGFP-target (Gag or Env). The scheme illustrates the target genes fused to the EGFP gene, the CMV promoter and the SV40 transcription termination signal. The fusion protein is coded by ORF EGFP-target (arrow). (B) HTLV-1 genomic organization and target genes *gag* and *env*. The arrows show the exact positions (in parentheses) of siRNAs used to inhibit both genes.

Table 2
siRNAs sequences used to inhibit *gag* and *env* genes and negative controls.

Gene targets	Name	Gene position	Sequences
gag and *env*	Negative control siRNA	–	Ambion Cat#4611
gag	siRNA Gag1	609–629	5′-GCAGCUAGAUAGCCUUAUATT-3′ 3′-TTCGUCGAUCUAUCGGAAUAU-5′
gag	siRNA Gag2	700–720	5′-CCACAACAACAAGGAUUAATT-3′ 3′-TTGGUGUUGUUGUUCCUAAUU-5′
gag	siRNA Gag3	968–988	5′-GCCCUCUAGGAGAUAUGUUTT-3′ 3′-TTCGGGAGAUCCUCUAUACAA-5′
–	siRNA Scr Gag	–	5′-CCAACAACAGGAUACAUAATT-3′ 3′-TTGGUUGUUGUCCUAUGUAUU-5′
env	siRNA Env1	236–256	5′-CCUAUUCCCUAUAUCUAUUTT-3′ 3′-TTGGAUAAGGGAUAUAGAUAA-5′
env	siRNA Env2	659–679	5′-GCACUAAUUAUACUUGCAUTT-3′ 3′-TTCGUGAUUAAUAUGAAGGUA-5′
env	siRNA Env3	1168–1188	5′-GGAGGAUUAUGCAAAGCAUTT-3′ 3′-TTCCUCCUAAUACGUUUCGUA-5′
–	siRNA Scr Env	–	5′-GCAAGAUCGAUUUCCGAUATT-3′ 3′-TTCGUUCUAGCUAAAGGCUAU-5′

hours before transfection, 2×10^5 cells were seeded to 80% confluence in 6-well culture plates with DMEM (2 ml/well). For the transfection of adherent HEK 293 cells, a total of 5 μg of reporter plasmid and 10 μl of siRNA (20 μM) mixed with SuperFect Transfection Reagent (Qiagen, Valencia, CA, USA) was used according to the manufacturer's instructions. The cells were incubated at 37 °C in the presence of 5% CO_2. Forty-eight hours after transfection, the expression of EGFP-target (*gag* or *env*) fusion proteins was observed directly under an inverted fluorescence microscope. This experiment was done in triplicate. Immunocytochemistry was also conducted to observe the expression of proteins.

2.4. Immunocytochemistry assay

Immunocytochemistry was performed to confirm the expression of EGFP-Gag/Env fusion proteins. Cells (5×10^4) were seeded to 80% confluency in 24-well culture plates with medium (1 ml/well), and transfections were carried out after 48 h. Forty-eight hours post-transfection, pEGFP-Gag/Env-transfected cells and non-transfected cells (negative controls) were fixed in 4% paraformaldehyde (Merck, Whitehouse Station, NJ, USA) for 20 min at room temperature, washed three times with PBS and then permeabilized with 0.2% Triton X-100 (Sigma–Aldrich, St. Louis, MO, USA) for 1 h at room temperature. The cells were blocked for 1 h with 5% bovine serum albumin (BSA) and 2% goat serum in PBS. Samples were incubated with primary antibodies anti-HTLV-I p19 (for Gag) and anti-HTLV-I gp46 (for Env) (Abcam Inc., Cambridge, MA, USA) at room temperature overnight. The secondary antibody used was Alexa Fluor 594 mouse antibody (Cat# A-21203, Invitrogen, Carlsbad, CA, USA). Nuclei were stained with DAPI (Vysis, Des Plaines, IL, USA). Cells were visualized using a confocal laser scanning microscope (CLSM) (LSM 710; Carl Zeiss, OberKochen, Germany) with in 63× objective lens in immersion oil, with a numerical aperture of 1.4. An argon laser of 590 nm was used to excite Gag or Env labeled with secondary antibodies, and emission was measured at 617 nm. EGFP fluorescence was excited with an argon laser at 488 nm, and the emission was measured at 525 nm. Visualization of the light scattering for each excitation wavelength was recorded in the multitracking mode using separate detection channels. Image analysis was carried out using CARL ZEISS ZEN 2008 software (Carl Zeiss). Control and test images were captured using identical settings.

2.5. Quantification of gag and env expression in transfected cells

2.5.1. Fluorescence microscopy

The fluorescence of EGFP-target in transfected HEK 293 cells was observed 48 h after transfection under an inverted fluorescence microscope (Olympus IX71, Tokyo, Japan) exciting the cells at 488 nm. Light microscope and fluorescence images in the same field were captured.

2.5.2. Flow cytometry analysis

After analysis by fluorescence microscopy, the cells were trypsinized, harvested and washed twice with phosphate-buffered saline, pH.7.5 (PBS). After centrifugation at 1200 rpm for 5 min at 4 °C, the cell pellet collected was resuspended in PBS to measure fluorescence using a BD FACSCalibur™ flow cytometer (BD Biosciences, San Jose, CA, USA) with filters (emission, 507 nm; excitation, 488 nm). Non-transfected HEK 293 cells were used as a control. The data collected by flow cytometry were analyzed with the CellQuest software (BD Biosciences). The mean fluorescence intensity of the cell population that exceeded the fluorescence intensity of control cells was calculated.

2.5.3. Real-time quantitative PCR

Forty-eight hours post-transfection, total RNA from transfected and non-transfected cells was extracted using TRIzol™ reagent (Invitrogen), analyzed for integrity by 1% agarose gel electrophoresis and treated with DNAse I Amplification Grade following manufacturer instructions (Invitrogen). One microgram of treated RNA was then reverse-transcribed into cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) according to manufacturer's recommendations. PCR amplification was carried out in 96-well plates with optical adhesives. The final reaction volume for the target reaction was 15 μl, consisting of 6.25 μl Taqman Universal PCR master mix (Applied Biosystems), 0.5 μl specific probe (FAM and MGB labeled, 5 μM), 0.625 μl of each specific primer (5 μM), 2 μl of cDNA template and 5 μl of nuclease-free water. The final reaction volume for the endogenous gene (GAPDH) reaction was 10 μl, consisting of 5 μl of Taqman Universal PCR master mix (Applied Biosystems), 0.53 μl of Human GAPD (GAPDH) Endogenous Control (VIC/TAMRA Probe, Primer Limited, part number 4310884E) from Applied Biosystems, 2 μl of cDNA template and 2.47 μl of nuclease-free water. For each run, standard cDNA, sample cDNA and no template con-

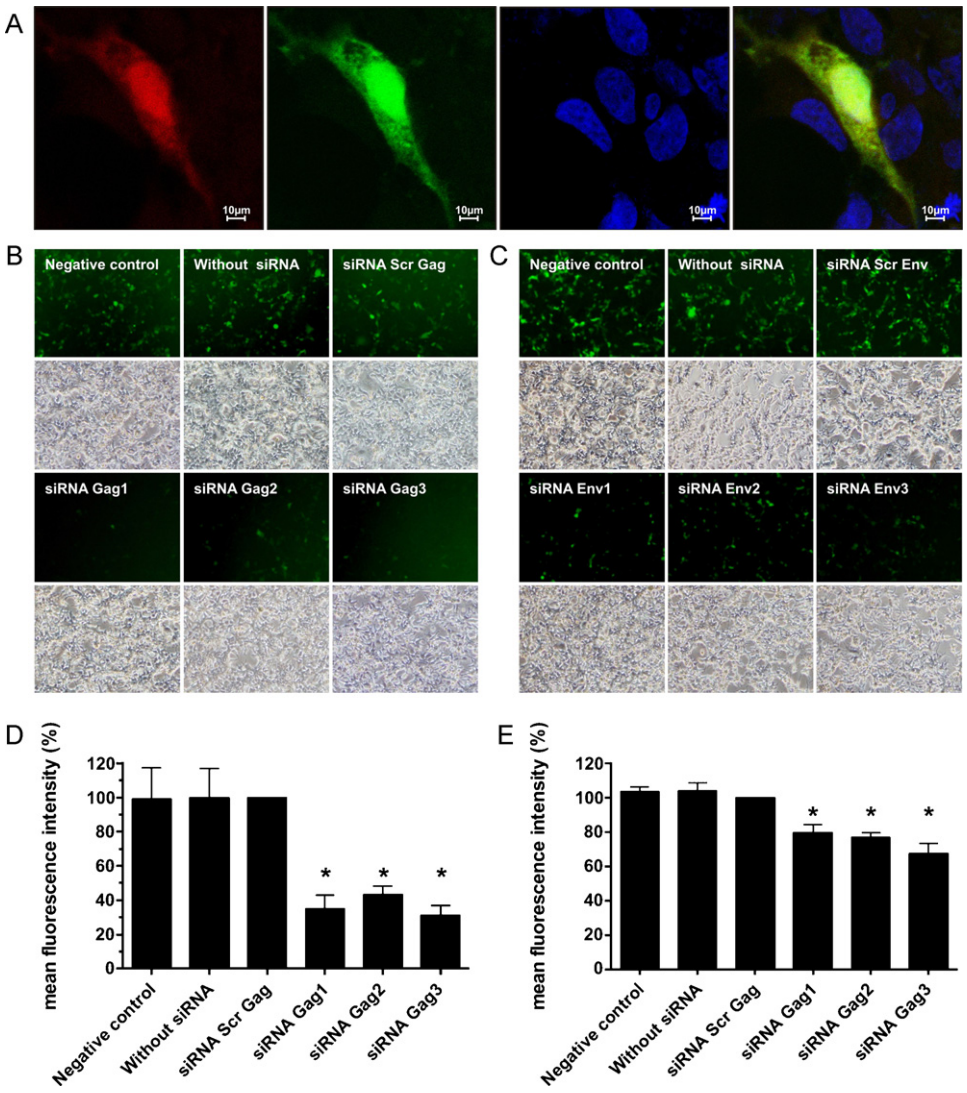

Fig. 2. EGFP-Gag and EGFP-Env expression in transfected HEK 293 cells. (A) Co-localization of Gag and EGFP in pEGFP-Gag transfected cells, observed by confocal microscopy. Nuclei were stained with DAPI. The cells transfected with pEGFP-Env presented the same profile (data not shown). (B) Fluorescence microscopy of HEK 293 cells co-transfected with pEGFP-Gag + negative control siRNA, pEGFP-Gag alone (without siRNA), pEGFP-Gag + siRNAs Scr Gag and siRNA Gag1, Gag2 or Gag3. (C) Fluorescence microscopy of HEK 293 cells co-transfected with pEGFP-Env + negative control siRNA, pEGFP-Env alone (without siRNA), pEGFP-Env + siRNAs Scr Env and siRNAs Env1, Env2 or Env3. The upper panels represent the cell fluorescence images recorded 48 h post-transfection and the lower ones represent the light microscopic view of cells in the same field (100×). (D) Mean fluorescence intensity of cells co-transfected with pEGFP-Gag and siRNAs, and transfected with pEGFP-Gag alone (without siRNA) by flow cytometry. Mean fluorescence was reduced significantly (*$p < 0.05$) in siRNAs Gag1, Gag2 and Gag3 compared to pEGFP-Gag and siRNA Scr Gag co-transfected cells. (E) Mean fluorescence intensity of cells co-transfected with pEGFP-Env + siRNAs, and transfected with pEGFP-Env alone (without siRNA) by flow cytometry. A significant reduction (*$p < 0.05$) was observed in siRNA Env1, Env2 and Env3 co-transfected cells compared to pEGFP-Env and siRNA Scr Env co-transfected cells. Means and standard deviations from three independent experiments are shown in (D) and (E).

trol were all assayed in duplicate. The reaction conditions were: 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C. The sense and antisense primers for the *env* gene were 5′-TCTAGTCGACGCTCCAGGATATG-3′ and 5′-CAGTTGGCTGGGTTCGGTAT-3′, respectively. The sense and antisense primers for the *gag* gene were 5′-CCCCCAAGTTCTTCCAGTCA-3′ and 5′-TGCCATGGGCGATGGT-3′. Both probes were marked with the 5′ reporter dye FAM and the 3′ quencher MGB, which were 5′-CCCCATCTGGTTCCT-3′ for the *env* and 5′-CCACATGGTGCCCC-3'for *gag*, respectively.

2.6. Statistical analysis

Data were reported as means ± SDs. Statistical analysis was performed by one-way analysis of variance (ANOVA), and the Dunnet multiple comparison test was applied to determine which means were significantly different ($p < 0.05$) from the control mean. All analyses were carried out using the GraphPad Prism software package (GraphPad software, San Diego, CA, USA).

3. Results

3.1. Expression of EGFP-Gag and EGFP-Env proteins

Before using the vectors constructed for the inhibition experiments, the expression of EGFP-Gag and of EGFP-Env was analyzed in transfected cells under a fluorescence microscope at various time points, post-transfection. Immunocytochemistry was also preformed to confirm the expression of fusion proteins. Both vectors expressed the fusion gene efficiently (Fig. 2A–C). The fluorescence at 48 h post-transfection was chosen for experiments with both constructs.

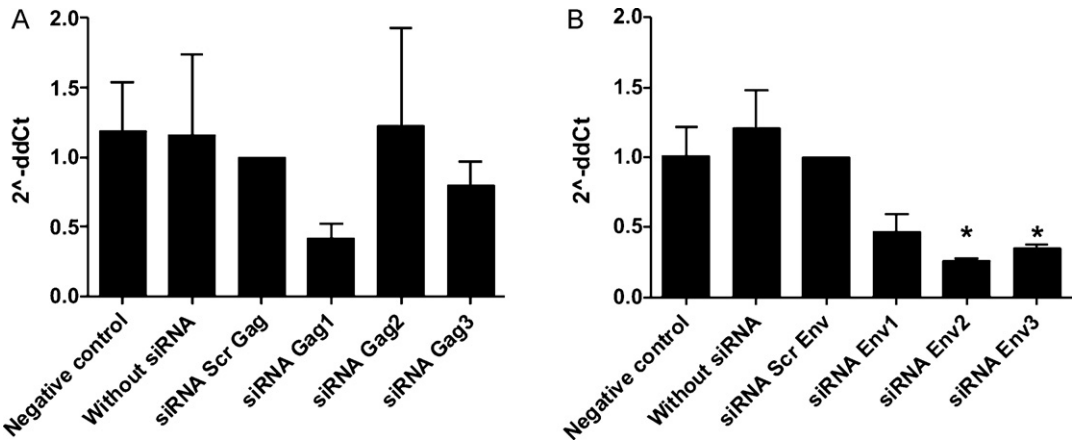

Fig. 3. Inhibition of EGFP-Gag and EGFP-Env expression detected by real-time quantitative PCR in HEK-293 cells 48 h post-transfection. (A) Mean relative expression of *gag* gene in cells co-transfected with pEGFP-Gag + siRNAs and transfected with pEGFP-Gag alone (without siRNA) compared to pEGFP-Gag + siRNA Scr Gag co-transfected cells. The differences in gene expression were not statistically significant. (B) Mean relative expression of the *env* gene in cells co-transfected with pEGFP-Env + siRNAs and transfected with pEGFP-Env alone (without siRNA), compared to pEGFP-Env + siRNA Scr Env co-transfected cells. Significant reduction ($^*p < 0.05$) of gene expression was obtained in cells co-transfected with siRNAs Env2 or Env3 and pEGFP-Env compared to siRNA Scr Env co-transfected cells. Means and standard deviations from three independent experiments are shown in (A) and (B).

3.2. Inhibition of Gag and Env protein expression

The silencing of GFP was confirmed by fluorescence microscopy and flow cytometry in at least three independent experiments for the *gag* and *env* genes. HEK 293 cells were observed 48 h post-transfection under a fluorescence microscope to determine the silencing effect of siRNAs on Gag and Env protein expression in cultured cells (Fig. 2B and C). The data showed no difference between cells transfected with pEGFP-Gag or pEGFP-Env alone and cells co-transfected with pEGFP-Gag or pEGFP-Env and scrambled siRNAs (siRNA Scr Gag or siRNA Scr Env). On the other hand, the cells co-transfected with siRNAs Gag1, Gag2 and Gag3 and pEGFP-Gag demonstrated less fluorescence than those co-transfected with pEGFP-Gag and scrambled siRNA (Fig. 2B). For the *env* gene, the fluorescence intensity of HEK 293 cells co-transfected with pEGFP-Env and negative control or scrambled was stronger than that cells co-transfected with pEGFP-Env and siRNAs Env1, Env2 or Env3 (Fig. 2C).

The inhibitory effects of siRNAs on the expression of EGFP were validated quantitatively by flow cytometry, as shown in Fig. 2D and E. EGFP down-regulation was quantified by assessing the mean fluorescence of EGFP-positive cells. No significant differences in scrambled siRNA co-transfected cells were detected when compared to cells transfected with pEGFP-Gag or pEGFP-Env alone. Mean fluorescence was reduced significantly by 65%, 57% and 69% in siRNAs Gag1, Gag2 and Gag3, respectively ($p < 0.05$), compared to pEGFP-Gag and siRNA Scr Gag co-transfected cells. Significant reductions ($p < 0.05$) of 21%, 23% and 33% were observed in siRNAs Env1, Env2 and Env3 co-transfected cells respectively, compared to pEGFP-Env and siRNA Scr Env co-transfected cells.

3.3. gag and env mRNA quantification

gag and *env* mRNA was quantified 48 h post-transfection by real-time quantitative PCR using specific primers and probes and GAPDH expression as endogenous control. Although no significant differences were observed, the mean results demonstrated that siRNAs Gag1 and Gag3 reduced *gag* gene expression by 58% and 20%, respectively, compared to siRNA Scr Gag co-transfected cells. No silencing effect was observed for siRNA Gag2 (Fig. 3A). When *env* gene expression was analyzed, the results showed a significant reduction ($p < 0.05$) of gene expression by 64% or 75% in cells co-transfected with siRNAs Env2 or Env3 and pEGFP-Env, respectively, compared to siRNA Scr Env co-transfected cells (Fig. 3B). The results demonstrated that siRNA Env1 reduced *env* gene expression by 53%, but this did not reach statistical significance.

4. Discussion

RNAi technology is accepted by the scientific community as a potential clinical tool against infectious diseases (Ma et al., 2007; Haasnoot et al., 2007). To test HTLV-1 *gag* and *env* gene silencing by RNAi, these genes were cloned downstream of the EGFP gene in a plasmid report system. Reproducible expression of fusion protein was observed after 48 h by fluorescence microscopy, providing an efficient reporter system for further experiments. This reporter system was co-transfected with siRNAs in HEK 293 cells. Based on the fluorescence data, flow cytometry and real time quantitative PCR, two siRNAs targeted to the HTLV-1 *gag* gene reduced efficiently gene expression by different amounts compared to the negative siRNA, scrambled siRNAs and controls without siRNA. The three siRNAs targeted to the *env* gene were effective, inhibiting gene expression compared to the control.

Different levels of inhibition can be justified since the efficiency of different siRNAs against the same target RNA can vary significantly. The characteristics of the target RNA and intrinsic mechanisms of siRNA itself play a role in silencing (Kurreck, 2009). One of the two strands can be assembled preferentially into the RISC depending on the stability of the two siRNA strands; if the complementary strand is not assembled into RISC, silencing would thus not be observed. Furthermore, the sequence of the target RNA could be inaccessible to the complementary strand, affecting the silencing (Schubert et al., 2005). These factors may partly explain the variation of gene suppression observed in this study. In addition, siRNA efficiency is strongly related to complementarity. siRNA may recognize and bind to the target sequence with incomplete homology, resulting in merely repression of translation without mRNA target degradation. This fact may explain the difference between the results obtained by flow cytometry and real-time PCR.

Despite the global spread of HTLV-1, and because of the scarce number of studies conducted on infected patients, it is difficult to estimate its prevalence, but approximately 15–20 million people are infected worldwide (Cooper et al., 2009). Most HTLV-1-infected individuals remain asymptomatic, but the virus is associated with severe diseases subdivided into neoplastic (ATLL), inflammatory syndromes (HAM/TSP and uveitis) and opportunis-

tic infections (strongyloidiasis, scabies, etc.). Current treatment of HTLV-1-related diseases is aimed primarily at reducing symptoms. Combination chemotherapy, allogeneic stem cell transplantation, molecular-targeted agents, nucleoside analogues and interferons have been reported as therapeutic strategies for these diseases, but with only short-term benefits and, in some cases, a short median survival (Verdonck et al., 2007; Yasunaga and Matsuoka, 2007; Ishitsuka and Tamura, 2008; Oh and Jacobson, 2008). The identification of new targets is an important part of drug development.

Many investigators are using RNAi to combat viral infections. The first study reporting the use of siRNA for the treatment of viral infection showed an effective and specific degradation of viral mRNA, as well as a resultant ablation of the specific viral protein (Bitko and Barik, 2001). The use of siRNAs for the treatment of HIV-1 infection, specifically directed against the *gag* gene (Novina et al., 2002), was reported subsequently. At the same time, studies have demonstrated the inhibition of the regulatory HIV-1 *rev* gene (Lee et al., 2002) and infectivity factors such as the *vif* and *nef* genes (Jacque et al., 2002).

The structural HTLV-1 gene *gag* is related to virion assembly and release (Le Blanc et al., 2001), whereas the Env protein is involved in the interaction between the surface receptor of the host cell and the virus (Manel et al., 2003). Considering previous studies showing efficient inhibition of HIV-1 structural proteins Gag and Env (Novina et al., 2002; Park et al., 2002, 2003), and the importance of the corresponding genes in HTLV-1 for its biology, in the present study, RNAi technology was utilized to determine its efficacy for the inhibition of HTLV-1 structural genes. In addition, few studies have used RNAi during HTLV-1 infection. This technique has been employed previously to demonstrate the importance of GLUT-1 in HTLV-1 cell entrance (Manel et al., 2003), some regulatory *tax* gene functions (Nomura et al., 2004; Qu et al., 2004; Hara et al., 2008; Jung et al., 2008; Hieshima et al., 2008), virus release (Blot et al., 2004) and host cell gene functions (Tomita et al., 2009). Most of these studies used siRNAs against genes of the host cells or the regulatory *tax* gene. No other report used siRNAs for HTLV-1 structural genes.

In conclusion, this is the first report that demonstrates the specific silencing of HTLV-1 *gag* and *env* genes by siRNA. The present findings could be useful for studies of *gag* and/or *env* gene function and provide a tool for the development of new therapeutic strategies for the treatment and prevention of development HTLV-1-related symptoms in infected individuals.

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