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Sp100 Interacts With HIV-1 Integrase and Inhibits Viral Integration*

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Abbreviations: Sp100, speckled protein of 100kDa; HIV-1 IN, HIV-1 integrase; ND10, nuclear domain 10; PML, promyelocytic leukemia; DAXX, death-associated protein; TTRAP, TRAF and TNF receptor associated protein; HP1, heterochromatin protein 1; SUMO, small ubiquitin-related modifier; SIM, SUMO-interacting motif; LEDGF/p75, human lens epithelium-derived growth factor; HSV, Herpes simplex virus; HCMV, human cytomegalovirus; EBV, Epstein-Barr virus; AD, adenoviruses.

Abstract Sp100 is a constitutive protein of nuclear domain 10 (ND10) and is ubiquitous in mammal cells. It is involved in many cellular processes such as transcriptional regulation and the cellular intrinsic immune response against viral infection. Using a yeast mating assay, we found that Sp100 can interact with HIV-1 integrase (HIV-1 IN). This interaction was verified by co-immunoprecipitation, and intracellular imaging revealed that Sp100 and HIV-1 IN partially colocalized. Furthermore, mutant variants assay indicated that the C-terminal 300～480 residues of Sp100 and the catalytic domain of HIV-1 IN were responsible for this interaction. Knocking down endogenous Sp100 with Sp100-specific siRNA increased HIV-1 IN-mediated integration. Conversely, overexpression of Sp100 by transient transfection decreased HIV-1 IN-mediated integration. This is the first time that Sp100 has been found to interact with HIV-1 IN and inhibit lentiviral vector integration. It reveals a new function of Sp100 as a HIV-1 IN-interacting protein and expands knowledge of the cellular defensive response to viral infection.

Key words Sp100, HIV-1 integrase, lentivirus, integration
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Lentiviral vectors are capable of infecting a wide range of dividing and non-dividing cells and integrating stably into the host genome, resulting in long-term expression of the transgene. In recent years, the best-characterized lentivirus, HIV-1, has been widely used as a gene delivery vehicle. The HIV-1 genome contains nine open reading frames encoding at least 15 distinct proteins involved in the cycle of infection and a number of cis-acting elements required at various stages of the viral life cycle. HIV-1 integrase (HIV-1 IN), which catalyzes the insertion of the viral DNA into the host genome and plays a role in reverse transcription and nuclear import, is crucial in productive lentiviral infection[1-3].

HIV-1 IN is a 288-amino acid protein which consists of three well-defined structural domains: an N-terminal domain (residue 1～49), a catalytic core domain (residue 50～212) and a C-terminal domain (residue 213～288). The N-terminal domain contains a highly conserved HHCC motif that chelates zinc and contributes to catalytic function and multimerization. The C-terminal domain contains an SH3-like fold and contributes to DNA binding, multimerization and

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integrase-reverse transcriptase interaction. The catalytic core domain contains an invariant DDE motif that forms the enzyme active site. It contributes to multimerization and binds with viral and host DNAs during integration[2–3]. HIV-1 IN can bind with different cellular and viral proteins and the integration activity is modulated by these interactions [4–5]. For example, human lens epithelium-derived growth factor (LEDGF/p75) can interact directly with HIV-1 IN, enhancing its solubility, protecting it from degradation, enhancing its chromatin binding ability and targeting it to the transcriptional active region [5]. Rev can control its nucleocytoplasmic shuttle and regulating the integration process[6–7].

Nuclear domain 10(ND10) are dynamic, spherical, macromolecular structures which represent accumulations of multiple cellular proteins and form distinct globular structures in the interchromosomal space of nucleus [8]. PML and Sp100 are the core constitutive component of ND10 and most of the other ND10 proteins are transiently present at ND10 under certain circumstances. Till now, ND10 has been linked to a variety of cellular physiological processes, including transcriptional regulation, DNA-damage response, chromatin organization and so on [9]. Interestingly, ND10 are also involved in cellular antiviral defense and this antiviral function is antagonized by viral regulatory proteins[10].

Our previous study found that two ND10 proteins, Daxx and TTRAP, interact with HIV-1 IN and influence lentiviral integration and reporter gene expression [11–12]. As a main constitutive component of ND10, Sp100 was first identified as an autoantigen from patients suffering from primary biliary cirrhosis [13]. Sp100 participates in many cellular processes, such as viral infection, gene transcriptional regulation, tumor suppression and apoptosis [14–18]. In this study, we attempt to determine the role of Sp100 in HIV-1 IN-mediated integration.

1 Materials and methods

1.1 Plasmid construction

All plasmids used are verified by sequencing analysis, and the primers used are listed in Table 1.

Table 1 Primers for constructing plasmids mentioned in Materials and methods

Plasmid	Sense primers	Antisense primers
pB42AD-Sp100	5′ cggaattcatggcaggtgggggcggc 3′	5′ gcggtgacctaatcttctttacctgaccctc 3′
pB42AD-Sp100(Δ29～152)up	5′ cggaattcatggcaggtgggggcggc 3′	5′ ggagaggaggaagatggttcatctcatttg 3′
pB42AD-Sp100(Δ29～152)down	5′ catcttcctcctctccaagaaagtgaagaag 3′	5′ gcggtgacctaatcttctttacctgaccctc 3′
pB42AD-Sp100(Δ153～286)up	5′ cggaattcatggcaggtgggggcggc 3′	5′ caggaattcaatttgtcatggattacattttc 3′
pB42AD-Sp100(Δ153～286)down	5′ gacaaattgaattcctgttctgtgcgactg 3′	5′ gcggtgacctaatcttctttacctgaccctc 3′
pB42AD-Sp100(Δ287～333)up	5′ cggaattcatggcaggtgggggcggc 3′	5′ cagtggaaatttggattccatggttgtgtag 3′
pB42AD-Sp100(Δ287～333)down	5′ ccaaatttccactgacgttgatgagccc 3′	5′ gcggtgacctaatcttctttacctgaccctc 3′
pB42AD-Sp100(Δ334～407)up	5′ cggaattcatggcaggtgggggcggc 3′	5′ ctggattctccttcagagtcctcactgc 3′
pB42AD-Sp100(Δ334～407)down	5′ ctgaaggagaatccagtgaggaggaggc 3′	5′ gcggtgacctaatcttctttacctgaccctc 3′
pB42AD-Sp100 1～300	5′ cggaattcatggcaggtgggggcggc 3′	5’- gcggtcgacctttttcctttttttatatccacc 3′
pB42AD-Sp100 300～480	5′ cggaattcaagccattttctaattcaaaag 3′	5′ gcggtcgacctaatcttctttacctgaccctc 3′
pLexA-HIV-1 IN	5′ cggatccgtttttttagatggaatagataaggcccaa 3′	5′ cctcgagatcctcatcctgtctacttgccacaatc 3′
pLexA-HIV-1 IN(1～49)	5′ cgggatccactttttagatggaatag 3′	5′ ccgctcgagggcttccccttttag 3′
pLexA-HIV-1 IN(50～212)	5′ cgggatccacatgcatggacaagtag 3′	5′ ccgctcgagttctttagtttgtatg 3′
pLexA-HIV-1 IN(213～288)	5′ cgggatccacttacaaaaacaaattac 3′	5′ ccgctcgagatcctcatcctgtctac 3′
pCMV-HA-Sp100	5′ cggaattctggcaggtgggggcggc 3′	5′ gcggtaccctaatcttctttacctgaccctc 3′
pCMV-HA-Sp100(1～300)	5′ cggaattctggcaggtgggggcggc 3′	5′ gcggtacccctttttcctttttttatatccacc 3′
pDsRed-Sp100	5′ cggaattctgatggcaggtgggggcggc 3′	5′ gcggtaccacatcttctttacctgaccctcttc 3′
pEGFP-HIV IN	5′ ccgctcgagatggctagtgattttaacct 3′	5′ cgggatccatcctcatcctgtctacttg 3′

The HIV-1 IN was amplified from the lentivirus packaging plasmid pCMVΔR8.9 and subcloned into pLexA (Clontech) between the *Bam*H Ⅰ and *Xho* Ⅰ sites to produce the plasmid pLexA-HIV-1 IN. Three truncation mutants, HIV-1 IN1～49, HIV-1 IN50～212 and HIV-1 IN 213～288, were cloned from pLexA-HIV-1 IN and inserted into pLexA between the *Bam*H Ⅰ and *Xho* Ⅰ sites to generate pLexA-HIV-1 IN(1～49), pLexA-HIV-1 IN (50～212) and pLexA-HIV-1 IN (213～288).

The ORF of Sp100 (AAA35537) was amplified from a human fetal brain cDNA library (Clontech) and was inserted into plasmid pB42AD between the *Eco*R Ⅰ and *Xho* Ⅰ sites to generate pB42AD-Sp100. Using overlap PCR methods, four deletion mutants, Sp100Δ29～152, Sp100Δ153～286, Sp100Δ287～333 and Sp100Δ334～407, were inserted into pB42AD between the *Eco*R Ⅰ and *Xho* Ⅰ sites to generate pB42AD-Sp100Δ29～152, pB42AD-Sp100Δ153～286, pB42AD-Sp100Δ287～333 and pB42AD-Sp100Δ334～407. The N- or C-terminus of Sp100 was amplified from pB42AD-Sp100 and inserted into pB42AD between the *Eco*R Ⅰ and *Xho* Ⅰ sites to generate pB42AD-Sp100(1～300) and pB42AD-Sp100(300～480).

The ORF of Sp100 was amplified from pB42AD-Sp100 and inserted into the plasmid pCMV-HA (Clontech) between the *Eco*R Ⅰ and *Kpn* Ⅰ sites to generate pCMV-HA-Sp100. The N-terminus of Sp100 was amplified from pCMV-HA-Sp100 and inserted into pCMV-HA between the *Eco*R Ⅰ and *Kpn* Ⅰ sites to generate pCMV-HA-Sp100(1～300).

The plasmid pEGFP-HIV-1 IN was constructed as described previously [11]. The ORF of Sp100 was amplified from pB42AD-Sp100 and inserted into the plasmid pDsRed-N1 (Clontech) between the *Eco*R Ⅰ and *Kpn* Ⅰ sites to generate pSp100-DsRed, which expresses an Sp100-DsRed fusion protein.

1.2 Yeast two-hybrid assay

A MATCHMAKER pLexA two-hybrid system was purchased from Clontech (USA). The interaction between Sp100 and HIV-1 IN and their interacting domain were detected by the yeast mating assay. Using EGY48 [p8opLacZ] transfected with seven pB42AD-based constructs of Sp100 (pB42AD-Sp100, pB42AD-Sp100Δ29～152, pB42AD-Sp100Δ153～286, pB42AD-Sp100Δ287～333, pB42AD-Sp100Δ334～407, pB42AD-Sp100 (1～300) and pB42AD-Sp100 (300～480)) and YM4271 transfected with four pLexA-based constructs of HIV-1 IN (pLexA-HIV-1 IN, pLexA-HIV-1 IN(1～49), pLexA-HIV-1 IN(50～212) and pLexA-HIV-1 IN(213～288)), the interaction was tested according to the standard yeast mating protocol provided by the manufacturer.

1.3 Cell culture

The 293T cells were obtained from ATCC and were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, 100 U/ml penicillin, and 100 U/ml streptomycin at 37℃ in a humidified atmosphere of 5% CO_2.

1.4 Co-immunoprecipitation

Co-immunoprecipitation was employed to verify the interaction between HIV-1 IN and the cellular protein Sp100. Cells were transfected with pCMV-HA-Sp100 and pEGFP-HIV-1 IN using Lipofectamine2000 (Invitrogen). At 48 h post-transfection, the cells were harvested and lysed with 1 ml lysis buffer (20 mmol/L Tris pH 7.5, 100 mmol/L NaCl, 0.5% NP-40, 0.5 mmol/L EDTA, 0.5 mmol/L PMSF, 0.5% protease inhibitor cocktail, Sigma). Two micrograms of DNase were added to 1 ml of each cell lysate and incubated for 0.5 h at 4℃. Ten micrograms of rabbit anti-HA polyclonal antibody (Santa Cruz) or mouse anti-GFP monoclonal antibody (Santa Cruz) were added to 1 ml of each cell lysate. After incubating with end-over-end mixing for 2 h at 4℃, 10 μl of pre-washed protein A/G Sepharose (Santa Cruz) were added to each extract and shaken overnight at 4℃. The immunoprecipitates were washed three times with lysis buffer. The pellets were then separated by SDS-PAGE and transferred to a nitrocellulose membrane. The blot was probed using a mouse anti-GFP monoclonal antibody (Santa Cruz) or a rabbit anti-HA polyclonal antibody (Santa Cruz) followed by a horseradish-peroxidase-conjugated anti-mouse/anti-rabbit antibody (Sigma), and the bands were visualized using an enhanced chemiluminescent immunoblotting detection kit (Amersham).

1.5 Intracellular localization of Sp100 and HIV-1 IN

To determine the subcellular locations of Sp100 and HIV-1 IN in 293T cells, we transfected pSp100-DsRed and pEGFP-HIV-1 IN with HIV-1 IN and Sp100, respectively. Cells were transfected with the plasmids mentioned above using Lipofectamine2000 (Invitrogen). At 24 h post-transfection, the cells were observed under a confocal fluorescent microscope (TSC SP2, Leica). The nuclei were stained with DAPI as a control. The fluorescence of GFP, RFP and DAPI

was observed at wavelengths of 488, 543 and 405 nm for excitation and 510, 582 and 461 nm for emission respectively.

1.6 Sp100 knock-down and overexpression assay

The endogenous expression of Sp100 was knocked down by transfection with two different Sp100-specific duplex RNAs: 5′ AACCATGGAATCCAAATTAAT 3′ (Qiagen) and 5′ TGCGACTGGTGGATATAAA 3′[14]. The effect of these two siRNA duplexes was estimated by quantitative real-time RT-PCR and Western blot. As a control, the following human non-silencing duplex RNA was used: 5′ UUAAGUAGCUUGGC - CUUGAdTdT 3′; (GeneChem, China).

For overexpression of Sp100, 293T cells were transfected with pCMV-HA-Sp100 or pCMV-HA-Sp100(1～300), and pCMV-HA vector were transfected as a control.

1.7 Lentivirus preparation and infection

Lentiviral vectors were generated by transient transfection in 293T cells using a three-plasmid system as previously described [11]. Virus-containing cell supernatants were collected 48 h post-transfection, cleared by low-speed centrifugation and stored at –80 ℃ . The viral titer was calculated by the Reed-Muench method[19]. For viral infection, 293T cells were incubated with virus-containing medium at an MOI of 0.5 for 4 h and then replaced with virus-free medium.

1.8 Detection of lentiviral integration and reporter gene expression

Seventy-two hours after lentivirus infection, the percentage of EGFP-expressing cells and its mean intensity were quantified by flow cytometry (BD). The percentage of GFP-positive cells in the total populations of infected cells represented the integration rate of the virus, and the mean GFP intensity was used as an indicator of viral gene expression. The HIV-1 IN-mediated integration efficiency was also determined by quantitive real-time ALU-PCR based on genomic DNA. The cellular genomic DNA was extracted with a genomic DNA purification kit (Qiagen). To measure the relative levels of lentiviral integration, genomic DNA was quantified by means of an Alu-LTR real-time nested PCR assay [20]. The primers used for the first round were: 5′ GGCTAACTAGGGAACCCACTG 3′ , 5′ TCCCAGCTACTGGGGAGGCTGAGG 3′ and 5′ GCCTCCCAAAGTGCTGGGATTACAG 3′. After an initial denaturation at 95℃ for 8 min, 12 cycles of denaturing (95 ℃ , 30 s), annealing (55 ℃ , 30 s) and extension (72 ℃ , 170 s) were carried out. Using Sybr-Green-based detection (TOYOBO Code No. QPK-201, 201T), one aliquot (1/500) of the initial PCR products was subjected to a second round of PCR amplification. The primers used were 5′ GCTAGAGATTTTCCA-CACTGACTAA 3′ and 5′ GGCTAACTAGGGAAC-CCACTG 3′, and a 100-bp fragment of the lentivirus vector was obtained. A pair of primers for β-actin, 5′ ACGAGGCCCAGAGCAAGAG 3′ and 5′ TCTC-CATGTCGTCCCAGTTG 3′, were used as a control[21]. C_t values were collected, and the relative lentiviral integration levels of the samples were calculated. The GFP reporter gene expression was detected using quantitative real-time RT-PCR as described previously[11].

2 Results

2.1 Interaction between HIV-1 IN and Sp100

The yeast-two-hybrid assay revealed that Sp100 interacted with HIV-1 IN. The interaction region on Sp100 is located within residues 300 ～480. The deletion of the ND10 targeting domain (residues 29～152), the HP1 binding site (residues 287～333) and the activation domain(residues 334～407) had no effect on the interaction. The interacting region of HIV-1 IN lies within the catalytic domain of HIV-1 IN (Figure 1a, b).

The interaction between HIV-1 IN and Sp100 was further verified by co-immunoprecipitation analysis. 293T cells were transfected with pCMV-HA-Sp100 and EGFP-HIV-1 IN. The cell lysates were treated with DNAse before co-IP experiment to exclude IP of DNA-protein complexes. The rabbit anti-HA antibody was able to precipitate EGFP-HIV-1 IN but not EGFP (Figure 1c). Likewise, reciprocal co-IP of HIV-1 IN indicate that EGFP-HIV-1 IN but not EGFP can precipitate Sp100 (Figure 1d).

To localize HIV-1 IN and Sp100 within the cell, EGFP-HIV-1 IN and Sp100-DsRed proteins were expressed in 293T cells by co-transfection with the plasmids pEGFP-HIV-1 IN and pSp100-DsRed. As shown in Figure 2, punctates of red fluorescent Sp100-DsRed fusion proteins were predominantly located in the nucleus, whereas the green fluorescent EGFP-HIV-1 IN fusion proteins spread throughout the cells and also accumulated in the nucleus as punctations. Bright red-and-green puncta indicate co-localization of these two fusion proteins.

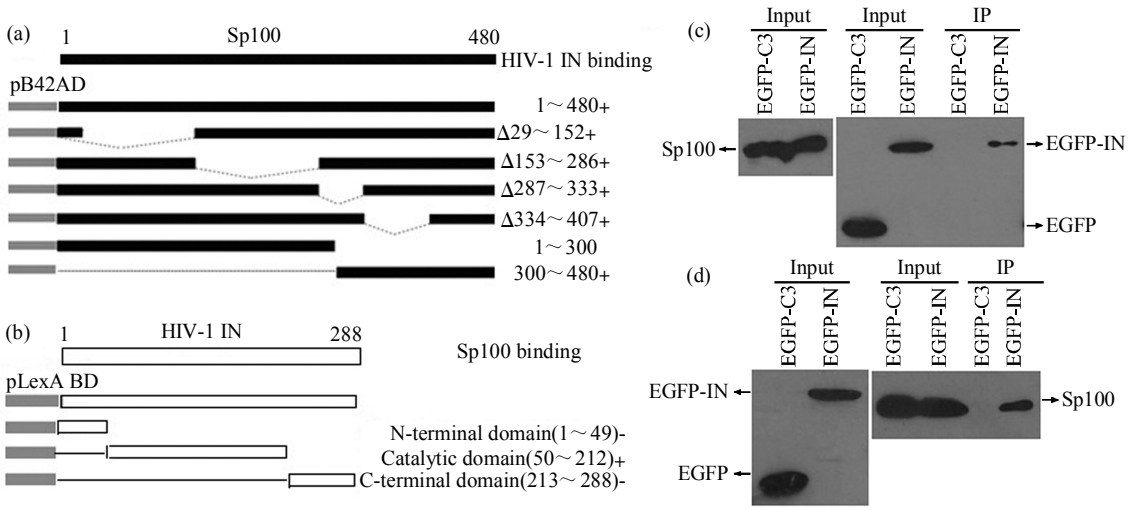

Fig. 1 Interaction between HIV-1 IN and Sp100

(a) Construction of Sp100 and its mutant pB42AD fusion plasmids for yeast mating assay. (b) Construction of HIV-1 IN and its truncation mutant pLexA fusion plasmids for yeast mating assay. (c) Lysates of pCMV-HA-Sp100 and pEGFP-HIV-1 IN transfected 293T cells were immunoprecipitated (IP) with a rabbit anti-HA antibody on protein A/G agarose beads. Samples were immunoblot (IB)-detected with a mouse anti-EGFP antibody. (d) Lysates of pCMV-HA-Sp100 and pEGFP-HIV-1 IN transfected 293T cells were immunoprecipitated (IP) with a mouse anti-EGFP antibody on protein A/G agarose beads. Samples were immunoblot (IB)-detected with a rabbit anti-HA antibody.

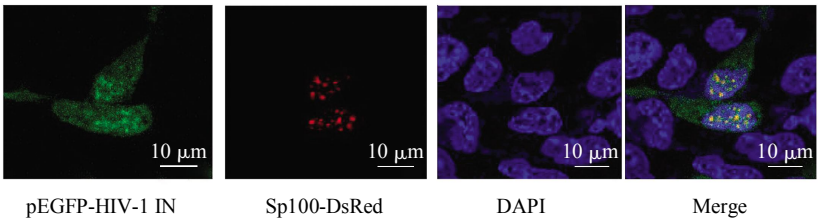

Fig. 2 Subcellular localization of HIV-1 IN and Sp100

pSp100-DsRed and pEGFP-HIV-1 IN were co-transfected into 293T cells. Expressed fluorescent Sp100-DsRed and EGFP-HIV-1 IN were observed under a confocal microscope. Nuclei were stained with DAPI (40×).

2.2 Endogenous Sp100 inhibited HIV-1 IN mediated integration

Because HIV-1 IN plays a crucial role in the lentivirus infection process, we studied the effect of the interaction between Sp100 and HIV-1 IN. Quantitative real-time RT-PCR and a Western blotting assay verified that two Sp100 specific siRNAs (siSp100a and siSp100b) can dramatically knock down endogenous Sp100 expression in 293T cells 24 h after siRNAs transfection (Figure 3a). Cells were transfected with either siSp100 or sicontrol for 24 h and then infected with the lentiviral vector at a MOI of 0.5. Three days after infection, the percentage of EGFP-expressing cells was measured by flow cytometry. As shown in Figure 3b, the percentage of EGFP-positive cells in the blank group (without siRNA) and siControl group (scrambled siRNA) were (21.17 ± 0.90)% and (23.48±1.62)%, whereas in Sp100 knock-down cells, the percentages rose to (48.08±1.28)% ($P < 0.01$) and (39.37±1.61)% ($P < 0.01$), respectively. Furthermore, an Alu-LTR real-time nested PCR array revealed that the relative levels of lentiviral integration in Sp100 knock-down cells were (1.48±0.12)% ($P < 0.01$) and (1.37 ±0.11)% times ($P < 0.01$), respectively, when compared with siControl cells (Figure 3c). Meanwhile, the effect of overexpression of Sp100 on HIV-1 IN mediated integration was also done. Cells were transfected with pCMV-HA-Sp100, Sp100 (1 ～300)

or pCMV-HA as a control (Figure 3d). As shown in Figure 3e, the percentage of EGFP-positive cells in the blank group and control group (pCMV-HA) were (21.17 ± 0.90)% and (20.82 ± 1.17)%, whereas in Sp100 overexpression cells, the percentages drop to (12.62±1.14)% ($P < 0.01$). Overexpression of Sp100 (1～300) which can not interact with HIV-1 IN, the percentage of EGFP-positive cells was (20.34±1.61)% ($P > 0.5$). Again, an Alu-LTR real-time nested PCR array revealed that the relative levels of lentiviral integration in Sp100 or Sp100(1～300) overexpression cells were (0.73±0.08)% ($P < 0.05$) and (0.95±0.09)% times ($P > 0.5$), respectively, when compared with control cells (Figure 3f). All these results suggested that Sp100 involved in HIV-1 IN mediated viral integration.

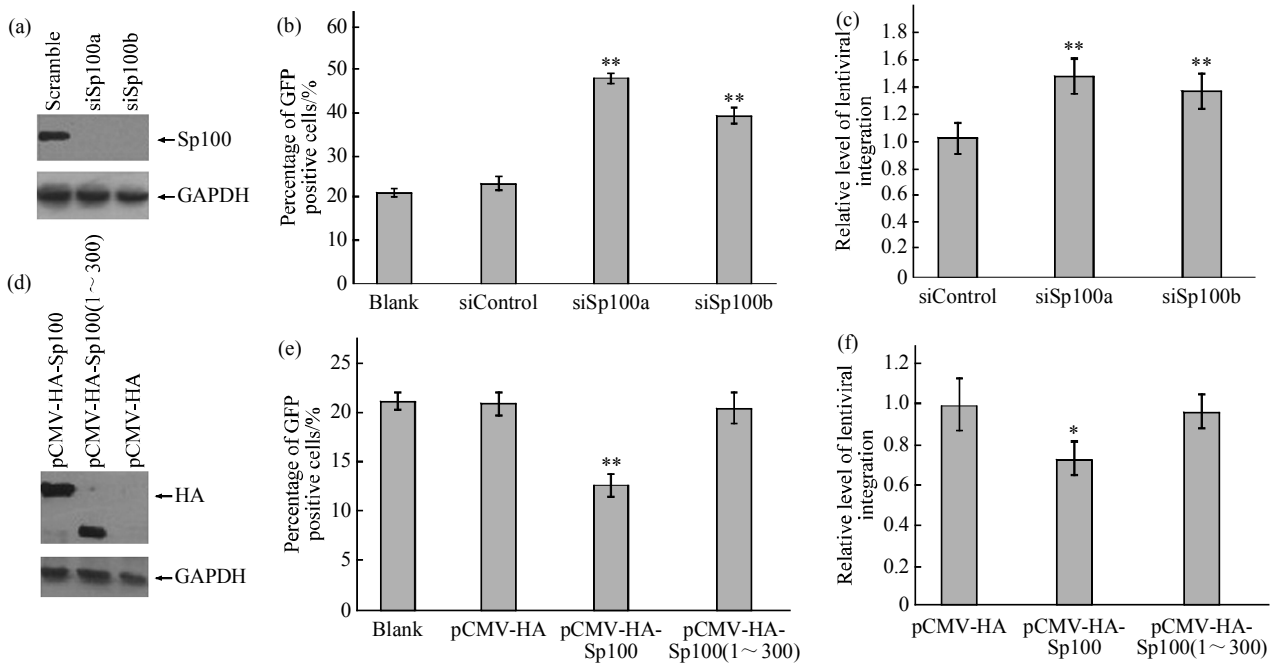

Fig. 3 Endogenous Sp100 inhibited HIV-1 IN mediated integration

(a) The knockdown efficiency of Sp100 was detected by Western blotting. (b) 293T cells were transfected with either siSp100 or a human non-silencing duplex RNA (siControl) and then infected by FUGW lentiviral vectors at an MOI of 0.5 24 h after transfection. Three days after infection, the percentage of GFP-expressing cells were detected by flow cytometry. $^{**}P < 0.01$ and $^{*}P < 0.05$. (c) 293T cells were transfected with different siRNA(as in (b)) and then infected by FUGW lentiviral vectors at an MOI of 0.5 24 h after transfection. Three days after infection, the genomic DNAs were extracted and the relative integration efficiency was measured by Alu-LTR real-time nested PCR. $^{**}P < 0.01$ and $^{*}P < 0.05$. (d) Overexpression of Sp100 or Sp100 (1～300) were detected by Western blotting. (e) 293T cells were transfected with pCMV-HA-Sp100, pCMV-HA-Sp100(1～300) or pCMV-HA and then infected by FUGW lentiviral vectors at an MOI of 0.5 24 h after transfection. Three days after infection, the percentage of GFP-expressing cells were detected by flow cytometry. $^{**}P < 0.01$ and $^{*}P < 0.05$. (f) 293T cells were transfected with different plasmids(as in (e)) and then infected by FUGW lentiviral vectors at an MOI of 0.5 24 h after transfection. Three days after infection, the genomic DNAs were extracted and the relative integration efficiency was measured by Alu-LTR real-time nested PCR. $^{**}P < 0.01$ and $^{*}P < 0.05$.

We also determined the impact of Sp100 on HIV-1 IN mediated reporter gene expression. Both the GFP mean intensity that was examined by a fluorescence-activated cell sorter (FACS) and the relative GFP mRNA level that was examined using a quantitative real-time RT-PCR assay showed only slight differences between the Sp100 knockdown/ overexpression cells and the sicontrol cells (data not shown).

3 Discussion

Sp100 is a core ND10 protein and plays a role in intrinsic cellular defense and gene expression regulation. Recently, Sp100 was shown to be involved in HSV and HCMV infection processes [17–18]. The present study is the first report of an interaction

between Sp100 and the lentiviral integrase HIV-1 IN, which results in the inhibition of lentiviral integration. The interaction between Sp100 and HIV-1 IN was revealed by a yeast mating assay and verified by co-immunoprecipitation. A subcellular localization assay also confirmed that these two proteins partially colocalize in the nucleus, which allows the possibility of interaction. To determine the interaction domain on Sp100, we constructed several Sp100 deletion mutants. Yeast mating tests showed that the C-terminal residues 300 ～480 which is an unidentified fragment was responsible for the interaction. The deletion of the ND10 targeting and homo-oligomerization domain in the N-terminus, the HP1 binding site and the activation domain in the C-terminus had no effect on the interaction [22-24]. We also constructed three HIV-1 IN truncation mutants and mapped the interaction domain of HIV-1 IN onto its catalytic core domain [2]. We previously reported that ND10 protein Daxx/TTRAP can interact with HIV-1 IN and this interaction can impact the reporter gene expression or facilitate the integration efficiency [11-12]. Daxx is recruited to ND10 through interacting with PML [25]. There is no report about interaction between Daxx/TTRAP and Sp100, and our experiment also showed no interaction between these proteins. What's more, no report suggests that there is an ND10 like complex in yeast. So we think it is more likely a direct interaction between Sp100 and HIV-1 IN.

HIV-1 IN plays an essential role in the viral life cycle. It catalyzes the stable insertion of viral cDNA into the host cell chromosome in two distinct steps: 3′-processing and 5′ strand transfer [1]. The catalytic core domain is indispensable for catalytic activity. It can bind directly with different cellular and viral proteins and these interplay regulate the integration process [4-7]. For example, chromatin association of HIV-1 IN is essential for HIV-1 integration in the integration process, but HIV-1 IN alone can bind to chromatin at a very low efficiency. However, interaction with LEDGF/p75 can tremendously promote HIV-1 IN's chromatin binding ability and target HIV-1 IN to the relevant cell compartment, thus lead to productive integration efficiency [5, 26]. Our experiment demonstrates the direct interaction between the catalytic core domain of HIV-1 IN and Sp100. Through this interaction alone, Sp100 may occupy the catalytic space and impede other viral and cellular proteins like Rev and LEDGF/p75 from interacting with HIV-1 IN, changing the relationship between HIV-1 IN and these proteins, thereby inhibiting the integration activity of HIV-1 IN.

HP1 is well-known as a silencing protein found at pericentromeres and telomeres. It plays a role in the organization of heterochromatin and gene silencing [27]. Sp100 can interact and colocalize with heterochromatin protein HP1. This suggests that Sp100 may function in chromatin organization and influence chromatin dynamics [24]. As chromatin construction plays a role in the integration activity and HIV-1 IN favors integration at active transcription units of host cell chromatin, the direct interaction between HIV-1 IN and Sp100 may locate the HIV-1 IN in a heterochromatin environment that is unfavorable to integration activity [28].

Through interaction with cellular proteins like LEDGF/p75, HIV-1 IN can be protected from proteosomal degradation and become increasingly stable [5]. It is known that one important function of ND10 is the elimination of exotic protein and maintenance of a normal cellular environment [8]. Indeed, lentivirus infection can impact the life cycle of host cells. We speculate that another way that Sp100 may inhibit HIV-1 IN-mediated integration is through the competitive binding to HIV-1 IN with other celluar proteins like LEDGF/p75 and the recruitment of HIV-1 IN into ND10, which may lead to the degradation of HIV-1 IN and prevent lentiviral gene integration.

By interacting with specific DNA-binding proteins, Sp100 participates in cellular transcriptional regulation [15, 24]. We wonder whether Sp100 can also impact the integrated reporter gene expression. GFP mean intensity and relative GFP mRNA levels both differed only slightly between the Sp100 knockdown cells and control cells. Since the GFP expression was observed only in those cells that did undergo HIV-1 IN-mediated reporter gene integration and activation, our research indicated that Sp100 had little direct effect on the reporter gene expression at either transcriptional or posttranscriptional level.

In summary, our study is the first to reveal a repressive effect of Sp100 on lentivirus integration efficiency through a direct interaction with HIV-1 IN. This finding expands our knowledge of Sp100 and its role in anti-viral defense. Sp100 binds to the catalytic domains of both HIV-1 IN and phiC31 integrase [29], which suggests to us that the interaction between

Sp100 and integrase may be an intrinsic property of Sp100. Because the integration and long-term expression of reporter genes are very important for gene therapy, our finding may shed some light on gene therapy research based on lentiviral vectors.

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Sp100 与 HIV-1 整合酶互作并抑制其介导的病毒整合 *

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摘要 Sp100 是核颗粒 ND10 的组成蛋白，在哺乳动物细胞中广泛存在．Sp100 参与多种细胞生理病理过程，如转录调控、细胞内抗病毒免疫等．利用酵母双杂交系统，我们发现了 Sp100 的互作蛋白 HIV-1 整合酶，免疫共沉淀实验进一步证实了 Sp100 与 HIV-1整合酶的互作，细胞内荧光共定位实验也证实了二者在细胞内部分共定位．此外，突变体实验表明，Sp100 的 C 端 300～480 氨基酸和 HIV-1 的催化结构域是两个蛋白质的互作区域．利用 siRNA 降低细胞内 Sp100 的表达量，可以增加 HIV-1 整合酶介导的病毒的整合，反之，细胞内过表达 Sp100 则会降低 HIV-1 整合酶介导的病毒的整合．这是首次发现 Sp100 可以和 HIV-1 整合酶发生相互作用，并进而抑制病毒的整合．我们发现了 Sp100 作为 HIV-1 整合酶互作蛋白的新功能，并扩展了细胞防御病毒感染的相关研究．

关键词 Sp100，HIV-1 整合酶，慢病毒，整合
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